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EVALUATION OF DEGRADING DEXTRAN HYDROGELS TO OBTAIN PULSED DRUG DELIVERY

STUDIE VAN DEGRADERENDE DEXTRAAN HYDROGELEN MET HET OOG OP GEPULSEERDE VRIJSTELLING VAN GENEESMIDDELEN

door

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DANKWOORD

Once upon a time,…

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CONTENTS

LIST OF ABBREVIATIONS

ADHD	:	attention deficiet hyperactivity disorder
BB	:	brilliant blue
CF	:	carboxyfluorescein
CHOL	:	cholesterol
CSLM	:	confocal scanning laser microscope
Dex-HEMA	:	dextran hydroxyethyl methacrylate
Dex-MA	:	dextran methacrylate
DMAEMA	:	dimethyl aminoethyl methacrylate
DOPA	:	diooleoyl glycero phosphate
DOPC	:	dioleoyl phosphatidylcholine
DOTAP	:	dioleoyl trimethylammonium propane
DS	:	degree of substitution
FITC	:	fluorescein isothiocyanate
GA	:	glycolic acid
GPC	:	gel permeatie chromatography
HEPES	:	hydroxyethyl piperazine ethane sulfonic acid
H-NMR	:	proton nuclear magnetic resonance
HPMC	:	hydroxy propyl methyl cellulose
IgG	:	imunoglobulin G
KPS	:	potassium peroxidisulfate
MA	:	methacrylate
MAA	:	methacrylic acid
MWCO	:	molecular weight cut-off
PB	:	phosphate buffer
P(CPP-SA)	:	poly(1,3 bis[carboxy phenoxypropane]-co-sebacic acid
PEG	:	poly(ethyleneglycol)
PLA	:	polylactic acid
SEM	:	scanning electon microscopy
SOPC	:	stearoyl oleoyl phosphatidylcholine
TEMED	:	N, N, N', N' tetramethylethylenediamine
TES	:	time-controlled exploding system
TRITC	:	tetra methyl rhodamine B isothiocyanate

LIST OF SYMBOLS

χ	:	Flory polymer-solvent interaction parameter
ε	:	permittivity
ϕ	:	functionality
η	:	viscosity
φ	:	polymer volume fraction
φ_e	:	equilibrium polymer volume fraction
λ	:	wavelength
μ	:	Electrophoretic mobility
ν	:	partial specific volume
π	:	pressure
π_{el}	:	elastic pressure
π_{osm}	:	osmotic pressure
π_{sw}	:	swelling pressure
ρ	:	density
τ	:	tensile strength
ω	:	weight
ξ	:	zeta-potential
c	:	concentration
G'	:	elastic modulus
k_{app}	:	apparent reaction rate constant
M_w	:	mean average molecular weight
M_n	:	number average molecular weight
n	:	scaling exponent
Q	:	swelling ratio
r	:	radius
R	:	correlation coefficient
R	:	universal gas constant
t	:	time
T	:	absolute temperature

GENERAL AIM OF THIS THESIS

Currently there is a growing interest in pulsed drug delivery systems, which release their drugs at programmed times. The general aim of this study is to evaluate a concept for pulsed drug delivery based on hydrogel microspheres (microgels) surrounded by a rigid semi-permeable membrane. Inside the microsphere a controlled degradation of the hydrogel polymer has to be found that results in an internal pressure build-up leading to a final pressure, which is sufficient to overcome the tensile strength of the surrounding membrane. When the membrane ruptures, all the drugs inside the microspheres must be liberated rapidly to create a pulse. This thesis is a first contribution to the search for a multiple pulsed drug delivery system schematically represented in Figure 1. A mixture of different types of self-exploding microparticles (e.g.: coated microgels bursting after one week, two weeks and three weeks, respectively) could be injected subcutaneous by a 'single shot'. As most vaccination schemes demand a repetitive contact with the antigen for complete vaccination, our concept is potentially interesting to obtain 'single shot vaccination'.

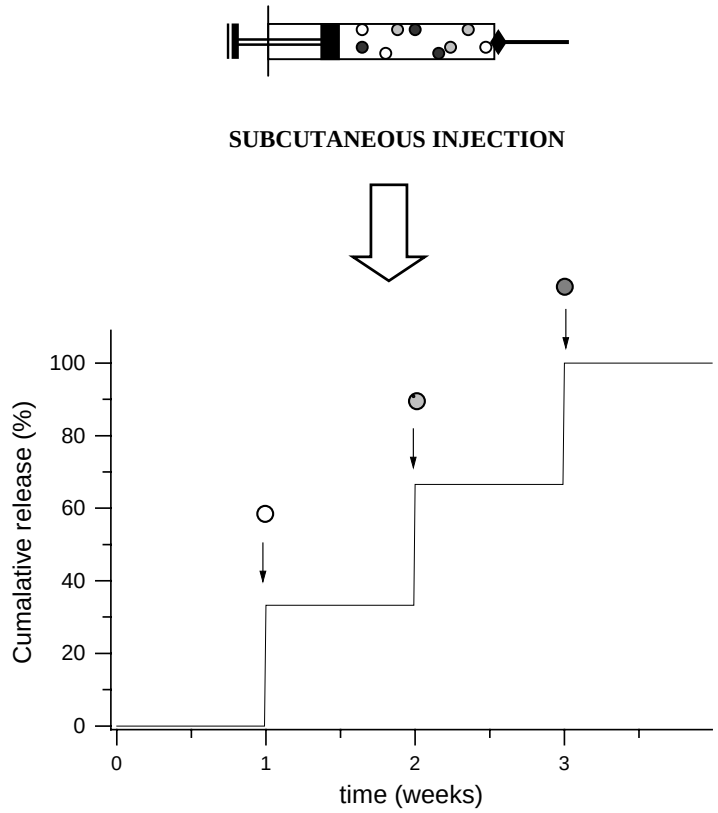


Figure 1. Drug delivery from coated biodegradable microgels which explode after different times resulting in multiple pulsed drug delivery after a single injection.

A real challenge is the exact timing of the rupture of the membrane creating the pulsed delivery of the drug. The strategy will be to determine what pressures are needed to rupture the membrane (depending on the size and composition of the microspheres, the thickness and strength of the membrane).

The first key aspect of this thesis includes the search for a degrading (non-coated) hydrogel with a predictable pressure profile as a function of time. The focus in this thesis is on dextran based gels. It will be necessary to understand the mechanism of pressure increase and swelling. Indeed, before surrounding a microgel with a membrane we must understand how the hydrogel degrades and what mechanism leads to the increase of internal pressure – taking into account that no or only partial swelling will occur when the hydrogel is surrounded by the membrane. Both thermodynamics and reaction kinetics determine this phenomenon. The thermodynamic properties include the swelling pressure (Π_{sw}) of the hydrogel which is described as the sum of two terms: an osmotic pressure (Π_{osm}) that expands the network and an elastic pressure (Π_{el}) that acts against expansion. The reaction kinetics of the hydrogels can be altered by changing the hydrogel compositions.

The second key aspect of this thesis includes the evaluation of the mechanical properties of the membrane (surrounding the hydrogel core). As membrane material we preferred lipids. We wonder which membrane should be used to obtain a unique membrane surrounded microgel system which is able to release the entrapped drugs by osmotic bursting of the membrane due to hydrogel degradation.

STRUCTURE OF THIS THESIS

Chapter 1 represents a literature overview on "programmed polymeric devices for pulsed delivery". The last part of Chapter 1 introduces the concept of 'self-exploding microparticles' consisting of a hydrogel core and a semi-permeable membrane. To make exploding microcapsules is the final goal of this work. As core material dextran based hydrogels are used. As membrane we especially focus on lipids.

Chapters 2-5 deal with the swelling pressure of dextran based hydrogels. As only a few references were found on swelling pressures of hydrogels, a method to measure the swelling pressure of degrading and non-degrading dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels is proposed in **Chapter 2**. The results are further evaluated in terms of scaling concepts.

In **Chapter 3** we wonder whether we can describe the swelling pressure profiles of the degrading dex-HEMA gels by using non-degraded dex-HEMA gels containing different amounts of free dextran (i.e. degradation product). The dex-HEMA/dextran gels mimick partially degraded dex-HEMA hydrogels. Swelling pressure profiles of dex-HEMA hydrogels with different compositions (degree of substitution and concentration at cross-linking) are also evaluated.

Because the osmotic deswelling method developed in Chapter 2 is very time and material consuming, a hydrogel osmometer is developed for the characterisation of the thermodynamic properties of degrading hydrogels. (**Chapter 4**) After validation of the osmometer, both methods for swelling pressure measurement of degrading hydrogels are compared.

Having established good methods for evaluation of the swelling pressure of degrading hydrogels (Chapter 2-4), the influence of the degradation mechanism on the swelling pressure is analysed in more detail. **Chapter 5** describes how the swelling pressure of dextran gels changes as a function of time in case the gels are degraded at their cross-links or at their back-bone, respectively. Dex-HEMA, with a degradation mechanism different to that of the dex-MA/dextranase hydrogels are included in this study. Differences in elastic modulus, release of degradation products, swelling and swelling pressure curves are shown and explained.

Chapter 6 deals with the mechanical properties of lipid membranes and is dealing with the final formulation: the 'self-exploding microparticles' (i.e. the lipo-beads). Membrane properties are evaluated by measuring the tensile strength of lipids with different compositions. Further a method for lipid coating of the dex-HEMA microgels is proposed. And, in the end, we show experimentally how degrading microgels can rupture their surrounding lipid membrane.

1

‘Programmed polymeric devices’ for pulsed drug delivery

Abstract

Pharmaceutical research strives to design drug delivery systems that respond to therapeutic needs. Considering the facts that physiological parameters (e.g. heart rate, blood pressure and plasma concentration of hormones, plasma proteins, enzymes) display constancy over time, drug delivery systems with a constant release profile have been designed. However, because of circadian rhythms in physiological parameters and pathological conditions (e.g. asthma, angina pectoris), the conventional paradigm concerning drug concentrations “the flatter the better” may not be what the organism may need. Instead, to correlate with our biological needs, 'precisely timed drug delivery', which could be accomplished with ‘programmable dosage forms’, is required. Precisely timed drug delivery may maximize therapeutic efficacy, may minimize dose frequency and may reduce toxicity by avoiding side effects and drug tolerance. This paper outlines the concepts that have been proposed to release drugs in a pulsed manner from pharmaceutical devices.

INTRODUCTION

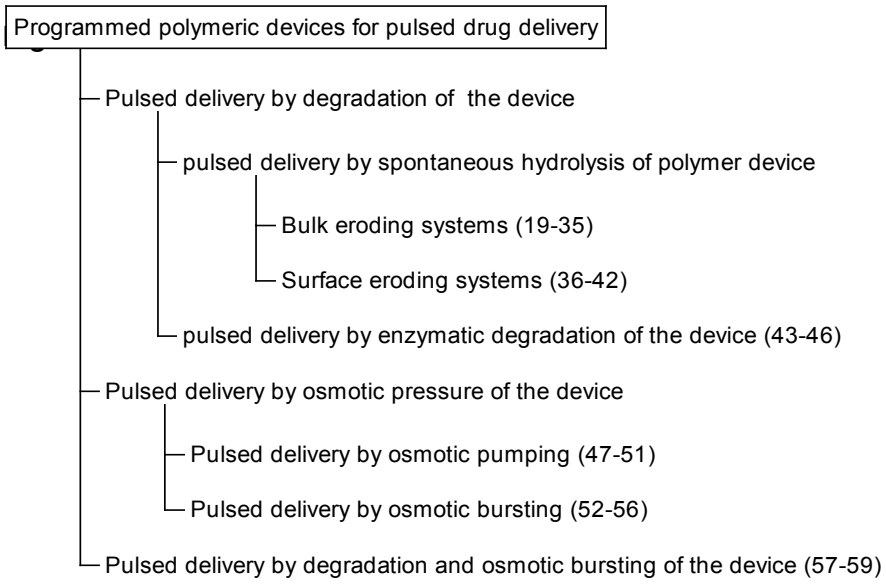
A common feature of many current controlled release devices is that they provide a continuous release over a prolonged period of time. However, there are many applications in medicine where a non-uniform release profile would be more beneficial.[1] For bioactive agents such as hormones, many have suggested that pulsed release may offer advantages over continuous release[2-4] as hormones are generally secreted by the body in a pulsed manner. Also, a pulsatile drug release pattern could be advantageous for drugs with an extensive first pass metabolism, for drugs that develop biological tolerance when they are constantly present at their target site and for drugs that require dosing at night. Also, devices that could give pulses of drug release at well-defined times after injection could be used to provide 'single-shot' vaccines where the initial and booster doses are contained in one delivery system. Such devices could improve vaccination coverage by reducing the number of vaccination sessions required to generate immunity.

'Pulsed drug release' is defined as the rapid and transient release of a drug after a predetermined off-release period. One way to classify 'pulsed drug delivery systems' is based on the physicochemical and biological principles that trigger the release. These devices are classified into 'programmed' and 'triggered' drug delivery systems. In 'programmed' delivery systems the release is completely governed by the inner mechanism of the device, i.e. the lag time prior to the drug release is controlled primarily by the delivery system. In 'triggered' delivery systems the release is governed by changes in the physiological environment of the device (biologically triggered systems) or by external stimuli (externally triggered systems).

Some examples of biologically triggered pulsed delivery systems include the delivery of insulin in response to glucose levels[5-7], the delivery of anti-inflammatory drugs in response to increased concentrations in hydroxyl radicals and hyaluronidase as may occur at inflammatory sites.[8-10]

In externally triggered systems external stimuli such as magnetism[11], ultrasound[12, 13] , temperature changes[14] , electrical effects and irradiation[15-17] activate the drug release. Because Kost and Langer have recently reported on pulsed delivery by biological and external triggers[18] , this manuscript aims to review pulsed drug delivery from programmed devices. We have focused especially on programmed drug delivery devices that are able to generate more than one pulse ('multiple pulse') and are of interest in treating diseases requiring repeated drug administration (see Sheme I).

Sheme I: Overview of the programmed polymeric devices for pulsed drug delivery.



Pulsed delivery by degradation of the device

This section outlines the delivery systems designed for pulsed release based upon the spontaneous hydrolysis or enzymatic degradation of the polymer comprising the device. The major idea to obtain pulsed release from such degradable polymer devices is that drugs (especially those with a higher molecular weight) can be physically entrapped in the non-degraded polymer matrix, and upon polymer degradation the matrix releases the drug. A combination of polymer matrices, one degrading faster than the others, in a single device may create opportunities to design multiple pulsed drug release systems.

Pulsed delivery by spontaneous hydrolysis of polymer devices

Bulk-eroding systems

D,L-polylactic acid (PLA) is the most established biocompatible polymer that undergoes bulk erosion in aqueous conditions by ester linkage hydrolysis. To alter the degradation rate of PLA, the polymer is co-polymerised with glycolic acid (GA). Cleland and co-workers[19-22] prepared microspheres with different PLGA compositions to achieve a 'single-shot' HIV-1 vaccine. In vitro release studies showed a substantial initial release of the antigen. After a lag of several weeks a continuous (not pulsed) release over a period of 4 weeks occurred.[19] PLGA microspheres were also investigated to achieve pulsed release of tetanus toxoid (M_w of 150 000 g/mol) and malaria antigens (M_w of 16 000 g/mol). [23-27]

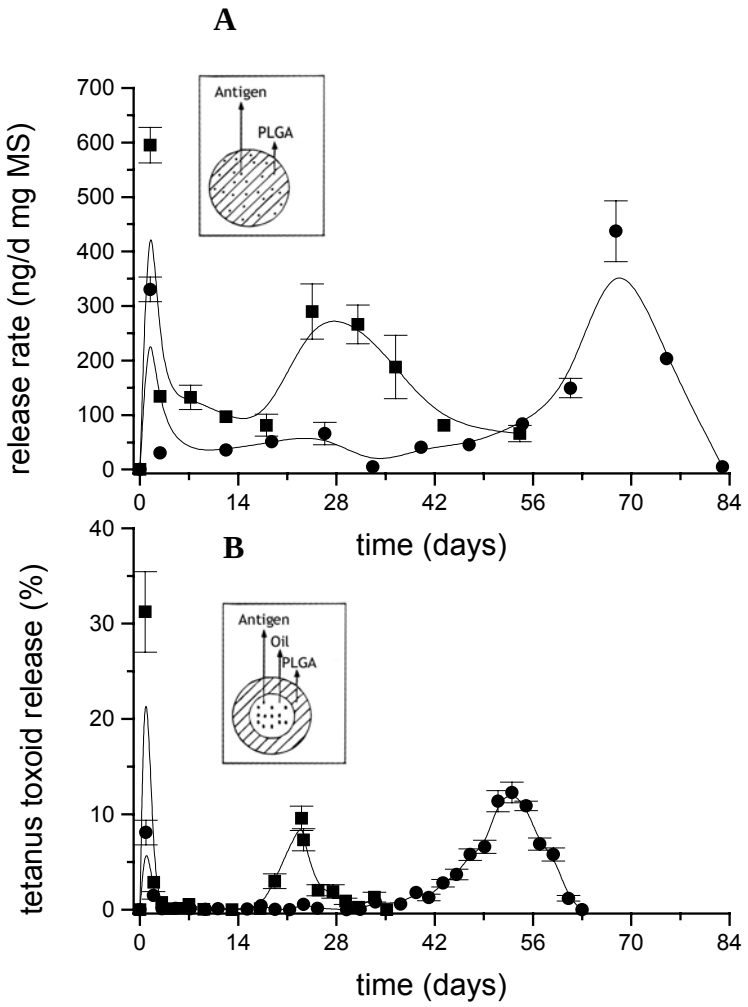


Figure 1A. In vitro release rates (in mg antigen per day and per mg microspheres) of tetanus toxoid antigen from spray-dried PLGA (50:50; (■)) and co-acervated PLGA (75:25; (●)) microspheres. [26] B. In vitro release of tetanus toxoid antigens from 'oil-filled PLGA microcapsules'. The lactic acid/glycolic acid ratio was 50:50 (■) and 75:25 (●), respectively. [31]

Figure 1A shows the typical in vitro release profile of tetanus toxoid antigens from PLGA microspheres.[27] It is characterized by an initial release, due to diffusion of the antigen near the surface of the microspheres, followed by a booster release after a certain lag phase that depends on the degradation rate of the matrix. Typically, the booster release occurs over a period of several weeks. Pulsed release is not obtained from PLGA microspheres. Moreover, PLGA shows some well-known disadvantages. Firstly, the exposure of the antigen to the acidic microenvironment inside the degrading PLGA-microspheres may degrade the antigen, (although basic additives like $Mg(OH)_2$ and $Ca(OH)_2$ may stabilize the antigens [28, 29]). And secondly, the encapsulated drugs, especially proteins, may react with erosion products (like glycolic and lactic acid monomers and oligomers).[30]

Sanchez et al. developed 'oil-filled PLGA microcapsules'[31] to overcome the PLGA limitations (as protein denaturation) described above. The microcapsules consist of an oil core, which contains the antigen, surrounded by an outer PLGA shell. As shown in Figure 1B, the time of tetanus toxoid release from the oil-filled microcapsules could be engineered by selection of an appropriate PLGA composition. The initial tetanus toxoid release could be substantially decreased by lowering the amount of GA. Also, by lowering the amount of GA the booster release occurred after 7 weeks instead of after 3 weeks. However, the booster release was once again not pulsed but continuous over several weeks.

Khoo and Thiel proposed PLGA based implants to obtain pulsed release of antigens.[32] The implant consists of a core of antigen in dibasic calcium phosphate coated with Eudragit S 100, which dissolves above a pH of 7.0. In turn, the Eudragit S 100 layer is coated with a blend of PLGA and ethyl cellulose. After hydratation of the outer layer, PLGA degrades and forms pores. Consequently, the Eudragit layer dissolves causing hydratation of the core and release of the antigen. The use of the Eudragit layer greatly delays the initial drug release. As an example, vitamin B12 was released after a lag time of 75 days. Co-administration of an uncoated and a coated implant resulted in pulsed release of antigens after respectively 1 day and 75 days, and allowed complete vaccination of an animal in a single handling.[32]

Pharmaceutical formulation of proteins under aqueous conditions is highly desirable to avoid protein degradation, which may occur when using organic solvents as in the preparation of PLGA devices. The group of Hennink introduced degradable dextran based microspheres, formed in aqueous environments, for pulsed delivery of proteins.[33] The dextran-hydroxyethylmethacrylate (dex-HEMA) microspheres degrade under physiological conditions due to hydrolysis of carbonate esters in the cross-links of the microgels. To obtain degradation controlled release and to avoid having the entrapped proteins passively diffuse from the microspheres, the initial mesh size of the dextran network is kept smaller than the hydrodynamic diameter of the encapsulated proteins. Figure 2 shows the in vitro release of IgG from dex-HEMA microspheres, which differ in cross-link density. The initial release was lower than 10% of the load. Increasing the cross-link density of the microspheres delayed the onset of release from 5 to 15 days while

the booster release occurred over a week, which is considerably shorter than observed for PLGA microspheres. To further lower the initial release and to enhance the loading of lipophilic drugs into the aqueous microspheres, drug loaded liposomes were entrapped in the dex-HEMA microspheres. In this way, intact drug carrying liposomes were released in a pulsed manner over a period of months. [34]

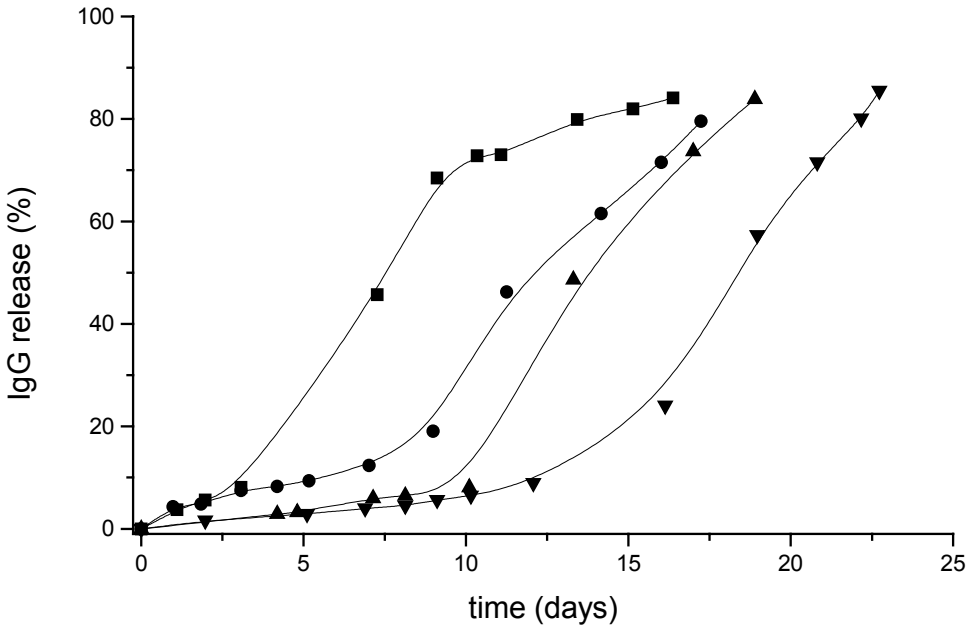


Figure 2. In vitro release of IgG from degrading dex-HEMA microspheres.[33] The dex-HEMA concentration of the microspheres was 50% (w/w), while the degree of substitution (i.e. the amount of HEMA groups per 100 glucopyranose units) equalled 3 (■), 6 (●), 8 (▲) and 11 (▼), respectively.

A possible disadvantage of the dextran-based microgels described above is the use of chemicals to cross-link the dex-HEMA. The group of Okano focused on calcium alginate beads that were prepared through physical cross-linking of an aqueous alginate solution[35] and which degrade (dissolve) due to exchange of chelated calcium ions with sodium ions from the medium. They showed that sterical entrapment in the gels, which is necessary to avoid initial release, could only be obtained for high molecular weight compounds (dextran $\geq$ 145 000 g/mol). Also, the lag time was short; it increased from 0.5 to 8 hours by increasing the alginate concentration and size of the beads. By combining calcium alginate beads of three different diameters, three pulses of dextran release could be established. However, the bead sizes were ≥ 1 millimetre. Much smaller sizes would be necessary, thus reducing the already short delay times.

Surface-eroding systems

Polyanhydride[36,37] and poly(ortho)ester[38] based matrices degrade by surface erosion because the hydrolysis of the polymer occurs faster than the water penetration into the matrices. Consequently, mass is lost more rapidly from the surface than from the bulk. This property may be attractive for pulsed delivery systems employing multi-laminated devices that consist of drug containing layers altered with (drug free) isolating layers. The length of both the lag times and the active delivery phases can be tailored by changing the type and thickness of the isolating and drug containing layers.

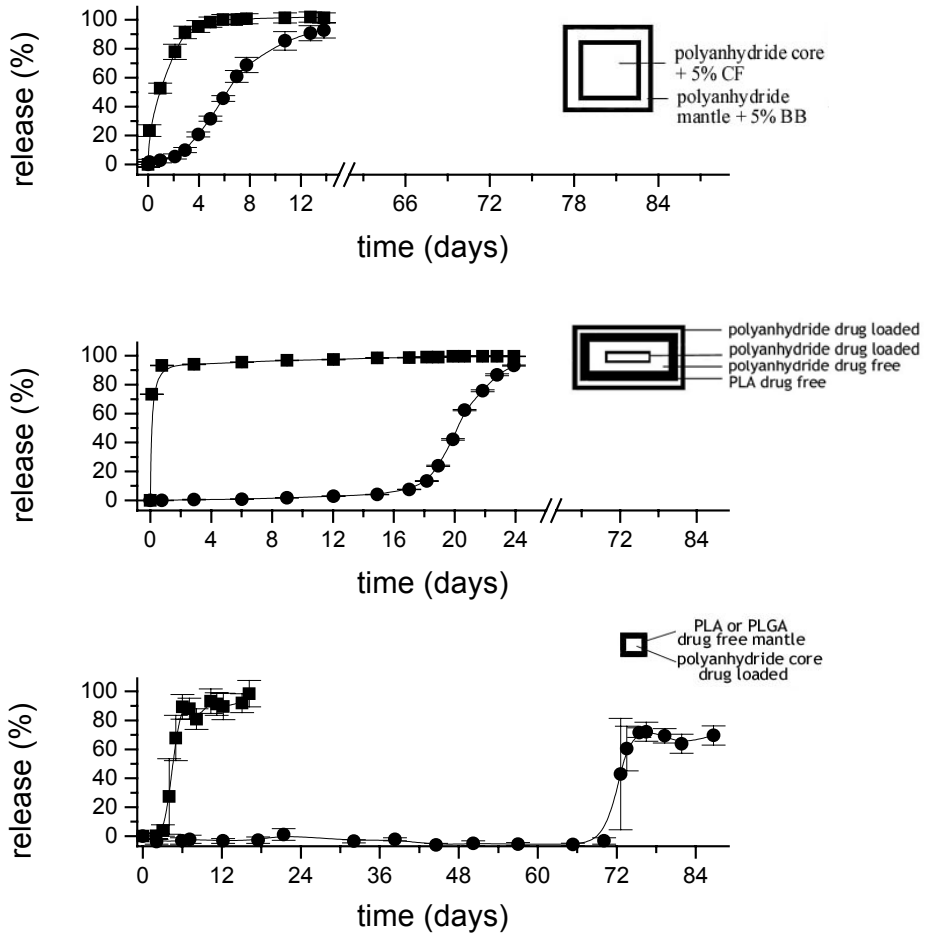


Figure 3A. Release of brilliant blue (BB; ■) and carboxyfluoresceïne (CF; ●) from a cylindrical polyanhydride device, which is schematically represented in the insert.[37] The core contains CF while the mantle is loaded with BB. B. Release of BB (■) and CF (●) from the implant as shown in the insert.[37] The core contains CF while the mantle contains BB. C. In vitro release of pyranine from the device shown in the insert. The PLGA ((■), M_w of 10 500g/mol) and PLA ((●), M_w of 30 000 g/mol) matrices are tempered.[37]

Göpferich and co-workers investigated the use of polyanhydrides in surface erodable implants.[37,39,40] The first reported implants consisted of a core and a mantle (Figure 3A), respectively 4 and 6 mm in diameter, both made of poly(1,3 bis[carboxy phenoxypropane]-co-sebacic acid) (p(CPP-SA)). As shown in Figure 3A, brilliant blue (BB), which was entrapped in the mantle, was released immediately while carboxyfluorescein (CF), present in the core, was released after two days, being too short for many applications. To overcome this, they suggested a combination of surface and bulk eroding polymer layers (Figure 3B); a drug-loaded p(CPP-SA) core, surrounded by a drug free mantle of p(CPP-SA) which was then coated with PLA to prevent early erosion and drug release from the core. Finally, a mantle of p(CPP-SA) was applied, containing the drug to be released initially. Two pulses occurred (Figure 3B). BB was released immediately while CF release, delayed by the erosion of the PLA-layer, started after two weeks. The rapid release of BB from the mantle was explained by its high water solubility. When the less water-soluble CF was in the outer layer, a pulse that lasted for about one week was observed. It indicates that the release from the outer layer strongly depends on the hydrophilicity and probably also on the molecular weight of the drug. The implants described above were intended to locally treat brain cancer. Again, a problem of these implants is their size, certainly when several implants need to be inserted. Moreover, the onset of drug release has to be adjusted from days to months. Therefore, the drug-loaded p(CPP-SA) core was directly coated with PL(G)A to trigger the onset of drug release.[39] Only 'tempered' PLGA and PLA matrices (i.e. matrices that were thermally treated in silicon oil to close pores and cracks) showed a pulsed release of pyranine in vitro with an onset time of release on day 4 and day 70, respectively (Figure 3C). Experiments on mice in which the matrices were subcutaneously implanted[39] suggested that the in vivo release is in reasonable agreement with the release results observed in vitro.

Jiang et al. have recently proposed another laminated device for pulsed protein delivery.[41] As the insert in Figure 4 shows, the cylindrical device consists of protein-loaded layers and isolating polyanhydride layers which govern the lag time between the pulses. The cylindrical device is surrounded by a polycarbonate coat and shows one open end. It was observed that the inner alternate layers gradually degrade from the open end. An interesting feature is that in the drug layer the protein is complexed to a polymer (i.e. poly(methacrylic acid)/polyethoxazoline). This complexation, which is pH-sensitive, promotes protein stability in the acidic microclimate created during polyanhydride degradation. As shown in Figure 4, at low pH the complexation retains the proteins in the drug layer until the upper drug free polyanhydride layer degrades totally. For myoglobine and FITC-BSA (Figure 4) sharp release pulses were obtained. Both the lag times and the duration of the pulses could be tailored by varying thickness and composition of the polyanhydride layers. Since the device is only useful for drugs that can be complexed to the polymer, Qiu et al. suggested using pH-sensitive degradable layers consisting of polyphosphazene.[42] The release profiles depend on the type of drug: the more hydrophilic the drug, the more easily it diffuses out, seriously influencing the intervals between the

pulses. Lag times could be tailored from 18 to 165 hours and the duration of the pulse varied between 19 and 40 hours. As a contrasting test, a device was examined that was composed of polyanhydride isolating layers and drug loaded PEG (not pH sensitive) layers. Similar to most other eroding devices mentioned above, an initial burst of the drug was followed by a sustained release phase instead of a second pulse. Therefore, the combination of polyanhydride layers with a pH-sensitive layer seems to be providing passively degrading devices for multiple pulsed drug release. The major disadvantage of these systems for single-shot vaccination is their large size (2-3 mm diameter and 11-14 mm height) and their complexity to manufacture (multi-layered).

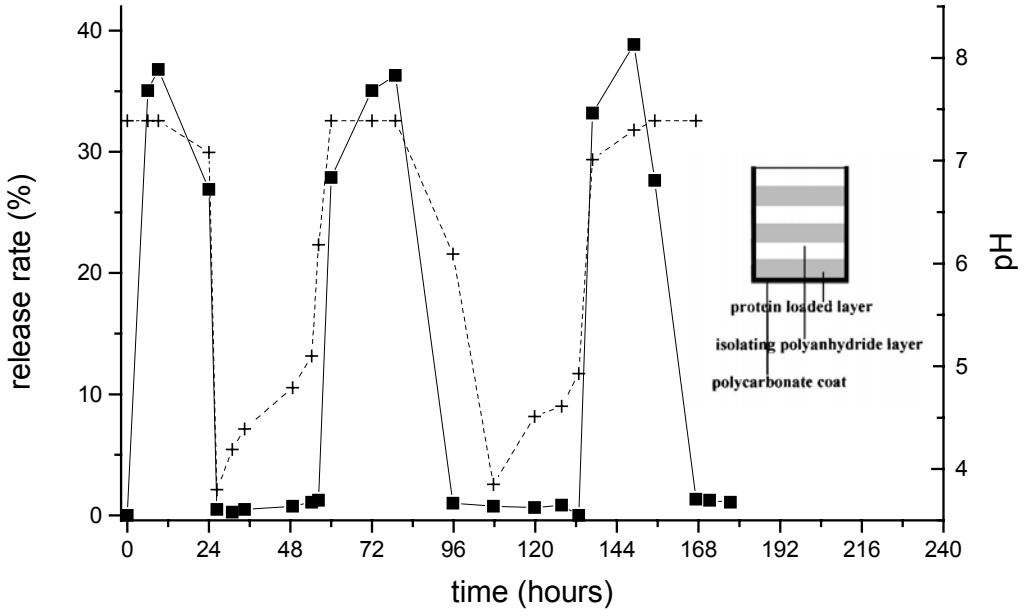


Figure 4. In vitro BSA release (■) from the laminated device (see insert) reported by Zhu et al.[41] In the protein layer the proteins are complexed to PMAA/PEO. The pH of the dissolution medium is also shown (+).

Pulsed delivery by enzymatic degradation of the device

Pharmaceutical matrices can also be degraded by incorporation of enzymes in the matrix during manufacturing. Franssen et al. studied enzymatic degrading dextran hydrogels.[43] The model protein IgG along with dextranase was incorporated during polymerisation of methacrylated dextran (dex-MA). The release of IgG was fully dependent on the degradation rate of the gel, which was strongly affected by both the concentration of dextranase and the cross-link density of the gel. At low dextranase

concentration a delay in the release was indeed observed, while at higher concentrations of dextranase, the release of IgG started immediately. To obtain multiple release pulses, the authors suggested simultaneously injecting micron sized dex-MA hydrogel particles with different amounts of dextranase and/or different compositions. However, miniaturisation of the gels seemed to be difficult since the release from dextranase containing microspheres followed zero-order kinetics without lag time.[44]

Kibat et al. designed another enzymatic degrading device.[45] Phospholipase A_2-coated liposomes were encapsulated in alginate microgels further coated with polylysine (see insert in Figure 5). Following hydratation, the phospholipase degraded the phospholipids, thus allowing release of entrapped drugs by diffusion through the microcapsule. As Figure 5 shows, a delay in the release of bovine serum albumin was observed and depended on the amount of phospholipase used. Pulsed release in vivo was demonstrated following subcutaneous implantation in mice[45]: the hydrogel matrix protected the liposomes from degradation and dispersion in the body, the liposomes acted as a drug depot, while the polylysine coat prevented dissolution of the alginate gel in the physiological environment.

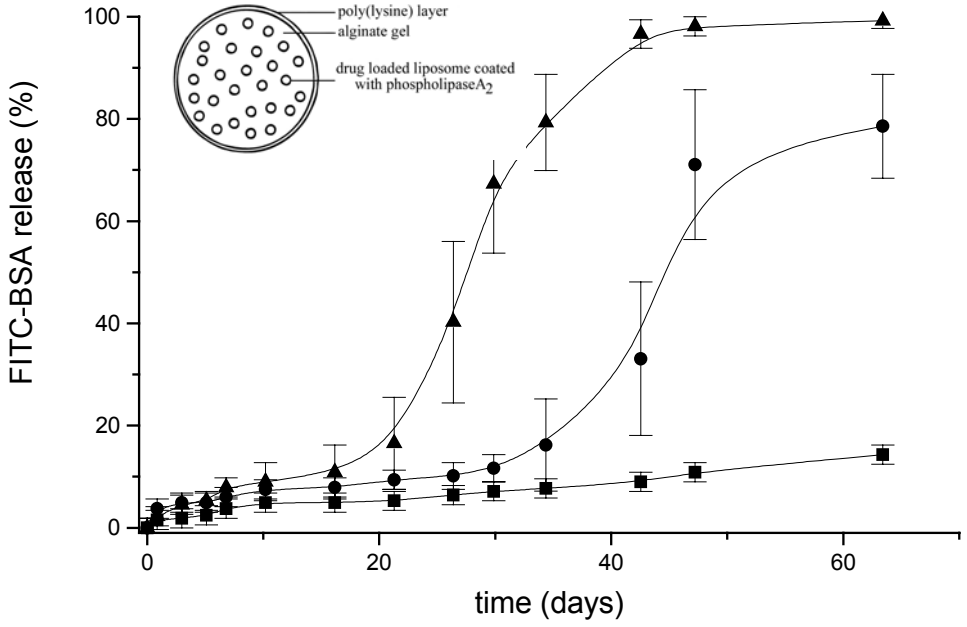


Figure 5. In vitro release of FITC-BSA from alginate-poly(lysine) microgels loaded with liposomes without phospholipase A_2 (■) and with phospholipase A_2 ((●) 1 Unit, (▲) 10 Units).[45]

Similar to the device shown in Figure 4 [41], Moriyama et al. described a non-degradable silicone tube containing alternating dextran layers and PEG grafted dextran layers loaded with insulin. [46] The PEG domains prevented diffusion of insulin into the dextran layers. In vitro pulsed insulin release occurred after respectively 10 and 50 hours due to surface degradation by the (non-physiological) dextranase that cleaves dextran from both open ends of the silicone tube. A similar device that could be degraded by hyaluronidase, which is present in higher concentrations at inflammatory sites, was also proposed.[8]

Pulsed delivery based on the osmotic pressure of the device

Establishing a pressure (osmotic and/or swelling pressure) in a pharmaceutical device requires a membrane surround the device that is permeable by water but impermeable by the drug and the pharmaceutical ingredients. Delivery from such devices is not only controlled by the osmotic or swelling agent, but also by the water permeability of the membrane, which in turn, is governed by the composition and thickness of the membrane.

Pulsed delivery by osmotic pumping of the device

Osmotic delivery systems have achieved wide acceptance as they can deliver drugs at a constant rate, and are largely independent from the environment.[47] However, researchers from Alza Corporation modified the osmotic pump to achieve pulsed delivery for circadian therapeutic requirements.

A veterinary pulsed delivery system (for implantation or oral intake) was derived from the Higuchi-Leeper pump.[48] Water diffuses through the semi-permeable membrane into the chamber containing a solution with excess solid salt. Due to the dissolution of the salt the osmotic pressure rises, which stretches the elastic cap. Once a critical pressure is produced, the orifice opens, releasing the drug as a pulse. The pressure then falls, the orifice closes, and the cycle repeats.

The elementary osmotic pump Volmax® is used to treat nocturnal asthma by delivering a pulse of salbutamol several hours after being swallowed.[49] Volmax® contains sodium chloride as the osmotic agent in the core. Sodium chloride also lowers the solubility of salbutamol. When the sodium chloride is expulsed after swallowing, the salbutamol quickly dissolves, which results in an abrupt increase in the release rate of salbutamol. Only a single pulse of the drug can be delivered by Volmax®. Moreover, the concept is only useful for delivery of drugs with specific solubility properties.

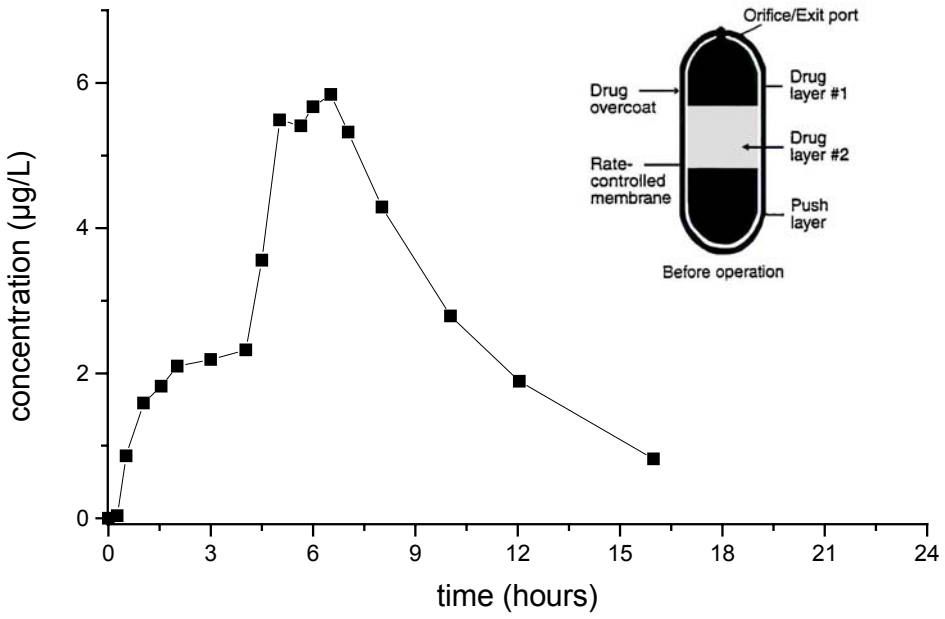


Figure 6. Blood concentration of methylphenidate hydrochloride after oral administration of a Concerta® (18 mg) capsule from which pulsed delivery is obtained by osmotic pumping.[51]

Concerta®, used to treat attention deficit hyperactivity disorder (ADHD) for school-aged children, is a modified push-pull osmotic pump which releases methylphenidate hydrochloride in a pulsed manner.[50,51] As shown in the insert of Figure 6, the Concerta® capsule comprises a tri-layer core surrounded by an immediate-release drug coat. The tri-layer core is composed of two drug layers and a push layer containing osmotic active components. In the gastro-intestinal tract, the drug coat dissolves within one hour, providing an initial dose of methylphenidate. Water can cross the semi-permeable membrane, hydrating the push layer and the interior drug layers. The push layer causes the release of methylphenidate through the precision laser-drilled orifice on the drug-layer end of the capsule. As Figure 6 shows, an initial plasma concentration is reached in 1-2 hours. Five to six hours after being swallowed methylphenidate hydrochloride is suddenly released.[51]

Pulsed delivery by osmotic bursting of the device

As early as 1975 Baker described the delivery of drugs by osmotic bursting of the device.[52] Figure 7A shows the device consisted of a water permeable membrane that enclosed the core containing the drug and an osmotic attractant, such as NaCl or other salts. In an aqueous environment water is osmotically drawn into the core, which swells until the membrane ruptures and releases the already dissolved drug in a 'single' pulse (Figure 7). This process, which is basically governed by the osmotic pressure of the core and the strength of the membrane, has been mathematically described by Kuethe et al.[53] The concept is comparable with the osmotic pump design; however, the device does not have an orifice in the outer membrane. This fact makes it less expensive because laser technology, which drills the orifice in the outer membrane, is not necessary.

Ueda et al. presented the 'time-controlled exploding system' (TES).[54] TES, which has a four-layer spherical structure (see insert in Figure 7B), consists of a polystyrene core (3.2 mm in diameter) on which the drug is loaded. The penetration of water through the water insoluble membrane (e.g. ethylcellulose) hydrates the swelling agent (e.g. hydroxypropylcellulose). The expansion of the swelling agent destroys the membrane and subsequent rapid drug release occurs. The authors showed that the lag time could be precisely programmed by the thickness of the outer membrane. Oral administration of TES particles with different lag times shows potential for short term (hours) multiple pulsed drug delivery (see Figure 7B). Since the pressure of the swelling agent destroys the outer membrane, the lag time is independent of the physicochemical properties of the encapsulated drug.

An osmotic bursting implant was reported by Thiel et al.[55] An antigen was included in a compressed core of Explotab® (sodium starch glycolate). The core was coated with a pH sensitive Eudragit S 100 film, which was again coated with an insoluble Eudragit NE30D film containing hydroxypropylmethylcellulose (HPMC) as a pore former. In an aqueous environment the HPMC in the outer coat dissolved, creating pores that allowed water to access the Eudragit S 100 coating (which dissolves at tissue pH). Consequently, the water enters the core, thereby swelling and rupturing the outer membrane, which results in a pulsed release of the antigen. In vitro, the release of antigens could be delayed for 14 to 26 days. Also in vivo pulsed antigen delivery has been achieved by co-administering coated and uncoated implants.

To achieve pulsed release of antigens Cardamone et al. packed alternate active and spacer tablets into a water impermeable tube (5 cm in length by 8 mm in outer diameter).[56] One end of the tube was plugged while the other end contained a swellable agent ('driver tablet') surrounded by a porous polymer cap. Water entered through the porous cap to swell the driver tablet, pushing the plug out of the tube. Hence, the surface of the first tablet was exposed to the environment.

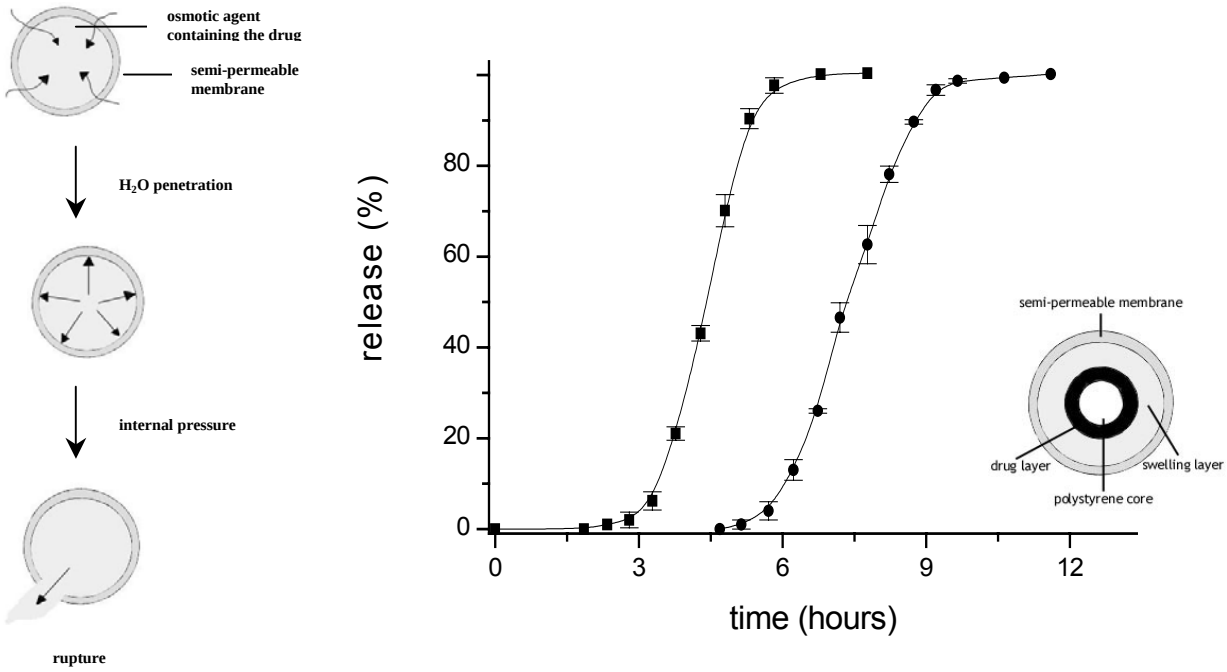


Figure 7A. Schematic representation of an osmotic bursting device when placed in a biological fluid. Water penetrates through the membrane. Due to the high solubility of the incorporated active agent, a high osmotic pressure is created, which ruptures the wall and leads to a sudden release of the drug. **B.** In vitro release profile of diclofenac sodium from TES (see insert) with a lag time of respectively 3 hours (■) and 6 hours (●).[60]

Pulsed delivery by degradation and osmotic bursting of the device

In the devices described above the increase in osmotic pressure, which governs the drug release, is mainly controlled by the rate at which water flows through the membrane. Rather recently devices have been reported where the degradation of the matrix determines the kinetics of osmotic pressure increase.

The group of Okano investigated the release from millimetre sized calcium alginate beads coated with a polyacrylamide layer (see insert in Figure 8).[57] Drug release occurs by sodium ions diffusing from the medium into the gel and exchanging with calcium ions. Consequently, alginate gels turns into alginate solutions increasing the osmotic pressure. This cracks the polymer coating resulting in a pulsed release of the contents (Figure 8). Only for high molecular weight dextran ($\geq$ 145 000 g/mol) could the initial release be suppressed to levels below 10% of the load. Applying thicker coats on the beads resulted in lag times of up to 60 hours. A fast booster release was observed.

Multiple pulsed deliveries of macromolecular drugs may be realized by mixing alginate beads with various coat thicknesses. However, the design of injectable micron sized alginate beads of this type could be a problem.

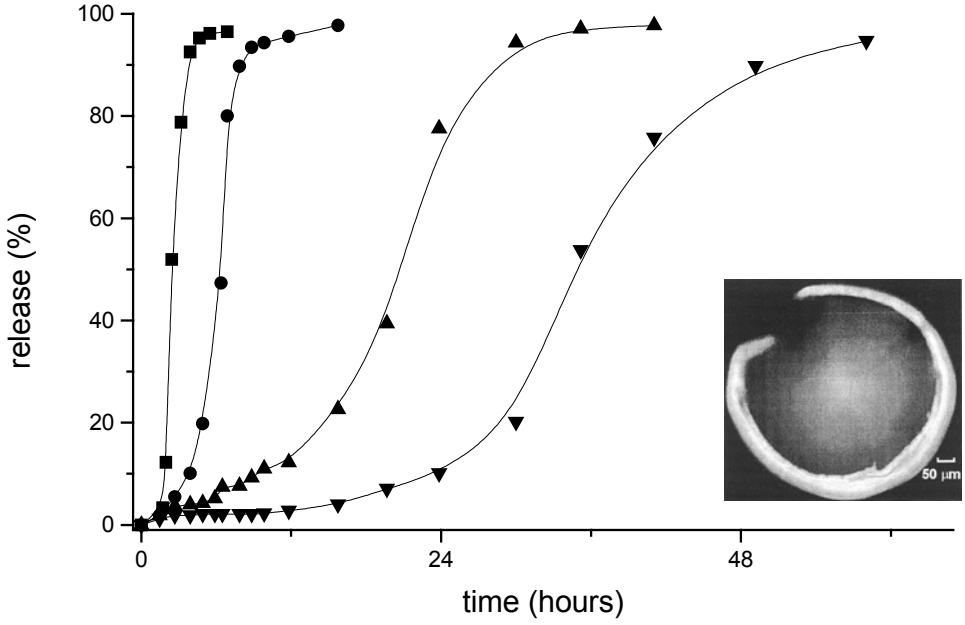


Figure 8. In vitro dextran (M_W of 145 000 g/mol) release from calcium alginate beads respectively without coating (■) and with a polyacrylamide coating of 25 (●), 50 (▲), and 75 (▼)µm thick.[57] The insert shows the crack in a coat surrounding the alginate bead (loaded with FITC-dextran) after 24 hours exposure to buffer.

Our group recently proposed a "degradation controlled exploding microcapsule" for pulsed delivery.[58,59] As in the concept of Okano, the capsule would consist of a water permeable membrane (allowing transport of water and preventing large molecular weight drugs from leaving the capsule) and an entrapped gel. However, the entrapped gel would be biodegradable. In contrast to the alginate beads, where the sodium ion flux determines the rate at which the alginate turns into a solution, the degradation rate of the gel (through spontaneous or enzymatic hydrolysis) would govern the osmotic pressure increase and thus the time of explosion of the microcapsules. While the onset of release is rather short for the polymer coated alginate beads, much longer lag times would be obtained in this way. The major challenge to realize this concept will be the coating of the degrading gel core. The membrane surrounding the gel has to be (i) very homogeneous, (ii) permeable by water, (iii) impermeable by the entrapped drug and the degradation products of the gel and (iv) rupture at the time the polymer gel turns into a polymer solution.

CONCLUSIONS

This review shows that, especially within the last decade, different concepts and devices have been proposed for precisely timed drug delivery. The list of potential uses of programmed devices for multiple pulsed drug delivery becomes longer as our knowledge of chronotherapy rapidly grows. Examples include the pulsatile administration of gonadotropin releasing hormone (for the induction of fertility), vaccines, corticosteroids (in the treatment of adrenal insufficiency) to name but a few. However, research on most of the programmed drug delivery devices is still in the 'concept phase'. Most systems are only for academic use, their performance in vivo has often not been tested while clinical results are clearly lacking. Obviously, the concepts and devices will have to be closely examined before they may be rendered applicable for practical use. Critical considerations are the biocompatibility of the pharmaceutical ingredients used, shelf life, ease and cost of large scale manufacturing, which appears to be complicated, requiring special equipment and many manufacturing steps.

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2

Swelling pressure observations on degrading dex-HEMA hydrogels

Abstract

The variation of the swelling pressure of dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels is determined as a function of the degradation time. In the first stage of the degradation process a moderate increase in the swelling pressure is observed due to the decrease of the elastic pressure. In this period the cross-link density of the polymer network gradually decreases but only a small amount of free polymer (dextran) is released. Towards the end of the degradation process, however, a sudden increase in the swelling pressure occurs which is accompanied by the release of a major amount of dextran chains. It is demonstrated that the chemical composition of the network (dex-HEMA content and the number of HEMA groups on the dextran chains) strongly affects the degradation rate of dex-HEMA hydrogels. These observations are important to design degrading hydrogel systems with tailored swelling pressure profile for pulsed drug delivery.

INTRODUCTION

Hydrogels are well suited for biomedical applications because of their tissue compatibility. *Non-degradable* hydrogels have been extensively studied as diffusion controlled and swelling controlled drug delivery devices[1,2]. Recently, there is a growing interest to evaluate *biodegradable* hydrogels for drug delivery applications[3-6]. Sustained drug release from a degrading hydrogel is obtained when the initial mesh size of the network is smaller than the size of the drug molecules, since the latter cannot leave the gel before the network has been degraded[1].

Currently, there is a major interest in pulsed drug delivery in which the pharmaceutical device releases the drug at a pre-programmed time[7-9]. Pulsed drug release can be achieved by creating a *rigid*, semi-permeable membrane around the degradable gel particle. The role of the membrane is twofold: (i) allows the transport of small molecules (e.g. water molecules, ions) between the gel and the surrounding solution, (ii) prevents large molecules (e.g., proteins, polymeric degradation products) from leaving the gel. During degradation the gel gradually liquefies and the swelling pressure Π_{sw} increases. When Π_{sw} exceeds the tensile strength of the membrane it ruptures[10], followed by a sudden release of the drug. The combination of different types of degradable coated gel particles could be of practical use in e.g. 'single shot vaccination' in which the initial and subsequent booster release of antigens could be obtained in one single injection. This avoids problems inherent with repeat immunizations and leads to an increased patient compliance.

Development of drug delivery devices with predictable release profiles requires the understanding of the thermodynamic and kinetic properties of the *degrading* hydrogel matrix. In an earlier paper we investigated the kinetics of degradation of dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels[11,12]. In this system degradation is caused by the hydrolysis of the carbonate ester link formed between the methacrylate group and the dextran molecule (Figure 1).

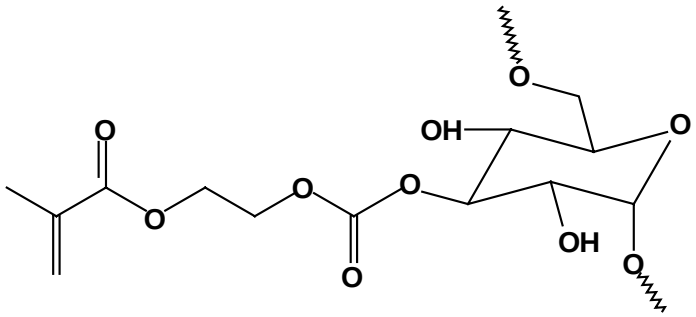

Figure 1. Chemical structure of the monomer in dex-HEMA, i.e. glucopyranose substituted with HEMA.

The aim of the present study is to investigate the effect of degradation on the swelling pressure of dex-HEMA hydrogels. Although the swelling behavior of polymer networks has been the subject of numerous investigations[13-19], according to our knowledge the variation of the swelling pressure in degrading gel systems has not been studied previously. Osmotic swelling pressure measurements are performed at different stages of degradation. The variation of the elastic modulus and the amount of free dextran produced in the degradation process are also measured. The results of the osmotic measurements are analyzed in the framework of the scaling theory.

EXPERIMENTAL SECTION

Dex-HEMA preparation and characterization

Dex-HEMA batches were prepared and characterized according to a method described elsewhere[20]. Dextran (Fluka, from *Leuconostoc ssp.*) with a molecular weight M_n of 19 000 g/mol was used. The degree of substitution (DS, the number of HEMA groups per 100 glucopyranose residues of dextran) was determined by proton nuclear magnetic resonance spectroscopy (H-NMR) in D_2O with a Gemini 300 spectrometer (Varian). The DS of the samples used in the present study were 2.9, 5.0 and 7.5, respectively.

Preparation and degradation of dex-HEMA hydrogels

Dex-HEMA gels were made by radical polymerization of aqueous dex-HEMA solutions. The solutions were prepared by dissolving dex-HEMA in a phosphate buffer (PB: 10 mM Na_2HPO_4, 0.02% sodium-azide, adjusted with 1 N hydrochloric acid to pH of 7.0). The polymerization reagents were N,N,N',N'-tetramethylene-ethylenediamine (TEMED; 20% v/v in deoxygenated PB, pH of 8.5) and potassium persulfate (KPS; 50 mg/mL in deoxygenated PB). 50 µL TEMED solution was added to 1 g polymer solution. After homogenization, 90 µL KPS solution was subsequently added to the system to initiate gelation. All containers were coated with poly(ethylene glycol) (PEG, 20 000 g/mol; 10% in PB) to reduce adhesion. Gelation required approximately 1 hour at room temperature. The hydrogel slabs used in the rheological measurements were made in cylindrical molds (diameter 23 mm, height 2 mm). For the other experiments gels were prepared in 2.5 mL polypropylene syringes (diameter 8.5 mm) from which the heads were sawn. After gelation the gel samples (~ 0.3 g gel) were removed from the syringe and cut with a thin wire. Degradation was studied in phosphate buffer (pH of 7.0) at 37 °C. Throughout this work the dex-HEMA concentration (%w/w) refers to the concentration at which cross-links were introduced.

Osmotic deswelling

In order to follow the swelling pressure generated by a confined hydrogel (i.e. when coated) as a function of degradation time, osmotic deswelling measurements were performed on dex-HEMA gels using a method described by Horkay and Zrinyi[16]. Gel specimens were surrounded by a semi-permeable membrane (Medicell dialysis bags, M_w cut-off between 12 000 and 14 000 g/mol). Similar dialysis bags were used in the purification step of the synthesis of dex-HEMA[20].

After different degradation times gel samples were equilibrated with PEG-solutions at 4 °C. PEG (Merck, M_w of 20 000 g/mol) was dissolved in citrate buffer (9.44 g/L Na_2HPO_4; 10.3 g/L citric acid and 0.2 g/L NaN_3, pH 4.4). The PEG concentration was varied in the range 0 - 12.5 g/100 mL. It was verified (from rheological measurements) that further degradation of the dex-HEMA gels did not occur during the osmotic deswelling measurements[12]. Equilibrium swelling was attained within 7 days. The reversibility of the swelling process was checked.

At equilibrium the swelling pressure of the gel is equal to the osmotic pressure of the PEG-solution. The osmotic pressure of the PEG-solution was calculated from the equation[21]

$$\Pi_{PEG} = \left[\frac{1}{M_n} + A_2\, c + A_3\, c^2\right] . c\, R\, T \times 10 \qquad (1)$$

where R is the gas constant, T is the absolute temperature, c is the PEG concentration (in g/100 mL), and A_2 and A_3 are the second and third virial coefficient, respectively. According to the data reported by Edmond and Ogston[22] for PEG (M_n of 20 000 g/mol), A_2=2.59 10^{-5} (mol.10^2 mL)/g^2 and A_3=1.35 10^{-6} (mol.10^4 mL^2)/g^3.

The dex-HEMA concentration of the gels was calculated using the relationship

$$c = \frac{w_{dex-HEMA}}{\left[\left(w_{dex-HEMA} \times v_1\right) + \left(\frac{w_e - w_{dex-HEMA}}{\rho}\right)\right]} \times 100 \qquad (2)$$

where w_e is the weight of the dex-HEMA gel, $w_{dex\text{-}HEMA}$ is the weight of dex-HEMA determined gravimetrically after drying the gel in a vacuum oven at 50 °C, ρ is the density of the buffer and v_1 is the specific volume of the dex-HEMA (v_1 =0.72 mL/g)[23]. The polymer volume fraction (φ) of the gels was calculated from the concentration of dex-HEMA and v_1.

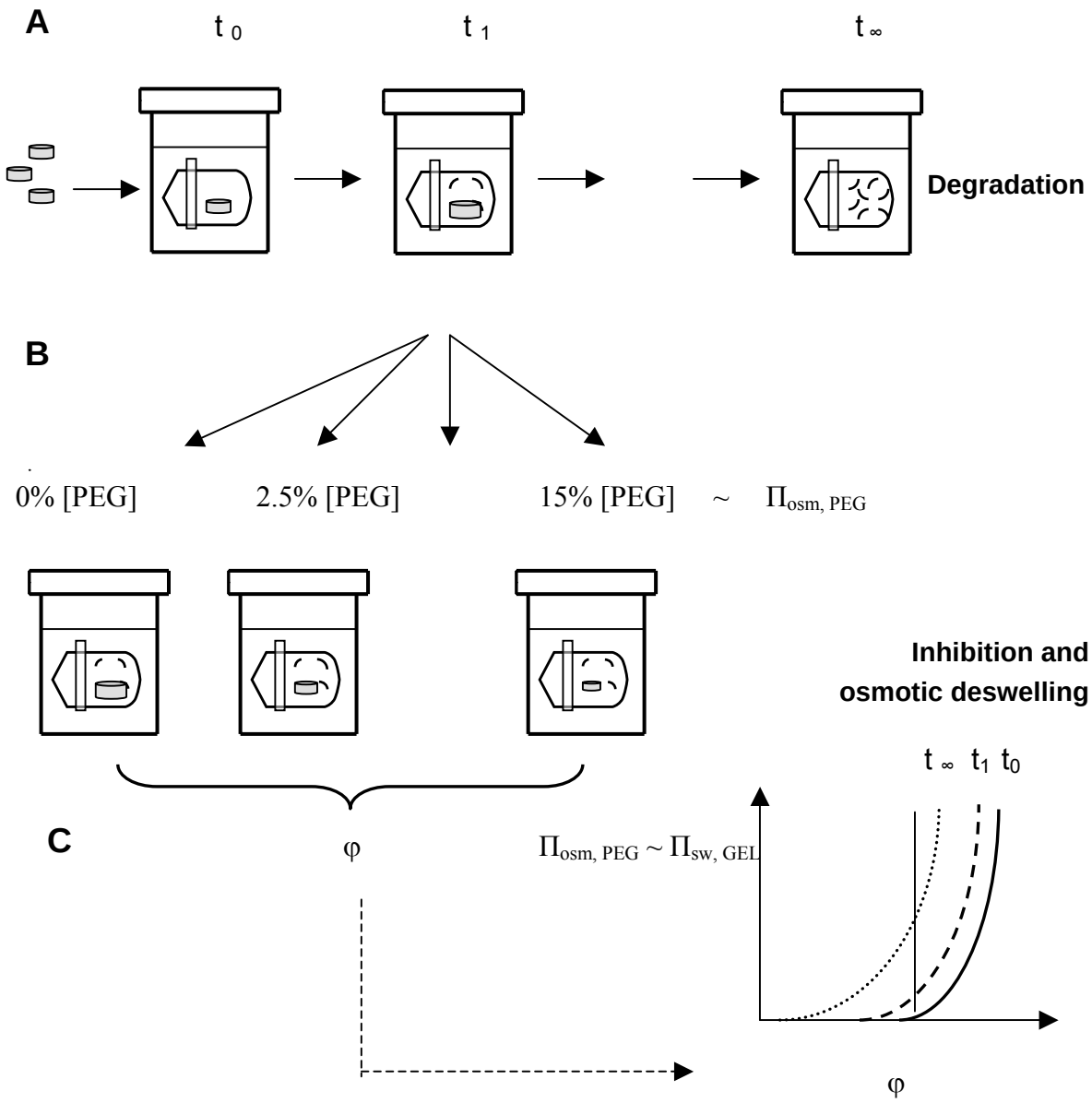

Figure 2. Schematic presentation of the osmotic deswelling measurements. **A.** After cross-linking, the gels are surrounded by a semi-permeable membrane. Dex-HEMA hydrogel degradation occurred at 37°C in PB and PH of 7.0. **B.** After well defined degradation times, the bags were immersed in PEG-solutions of known concentration. Degradation was inhibited (4°C and pH of 4.4). **C.** After 7 days equilibrium swelling was attained and the polymer volume fraction (φ) was determined gravimetrically. The swelling pressure profiles are obtained by plotting the osmotic pressure of the PEG-solutions versus the polymer volume fraction of the (partially degraded) gels. The vertical line represents the pressure generated by a confined hydrogel (i.e. having φ= constant) during degradation.

Mechanical characterization of degrading hydrogels

Rheological measurements were performed using an AR1000-N controlled stress rheometer from TA-Instruments. To avoid slippage the acrylic top plate was covered by sandpaper (diameter 2 cm). The bottom plate was replaced with a Plexiglas® plate with a roughened surface. Measurements were done in oscillation mode at 1 Hz in the linear viscoelastic region of these gels by applying a constant strain of 0.5%. After the measurements the hydrogel slabs were transferred into PB and stored at 37 °C. Further details of this method were reported by Meyvis et al[24].

Determination of free dextran chains in dex-HEMA gels

The concentration of the free dextran in the dex-HEMA hydrogels was determined from a release experiment performed in phosphate buffer at 37 °C. The amount of dextran chains in the solution was measured by gel permeation chromatography (GPC). The system consisted of a high pressure pump (Waters M510), an injector (Waters U6K) and a differential refractometer (Waters 410). 250 µL sample was injected and a flow rate of 0.5 mL/min was applied. The dex-HEMA concentration was calculated from the height of the peak using a calibration curve (concentration between 0 and 2.5 mg/mL) obtained for the corresponding dex-HEMA (DS2.9, DS5.0 and DS7.5). In the concentration range explored here the reproducibility of the GPC measurements is excellent (the correlation coefficient for linear regression exceeds 0.996 for each standard line).

RESULTS & DISCUSSION

Figure 2 shows the variation of the concentration of free dextran in different dex-HEMA hydrogels as a function of the degradation time, t. The dextran concentration, calculated from the amount of dextran released from dex-HEMA gels, refers to the initial volume of the (undegraded) gel. In principle, this concentration corresponds to the concentration of free dextran molecules in degrading gel particles surrounded by a rigid semi-permeable membrane. It appears in Figure 2 that first the sol fraction (unreacted dex-HEMA chains) leaves the gel. This feature is independent of the degradation process. In the second region (delay region) a relatively small amount of dextran is released. Finally, when the majority of the cross-links are cleaved the liberation of dextran chains is significantly enhanced. The delay in the release of dextran is expected since a chain can only become free when all cross-links connecting it to the network are cleaved.

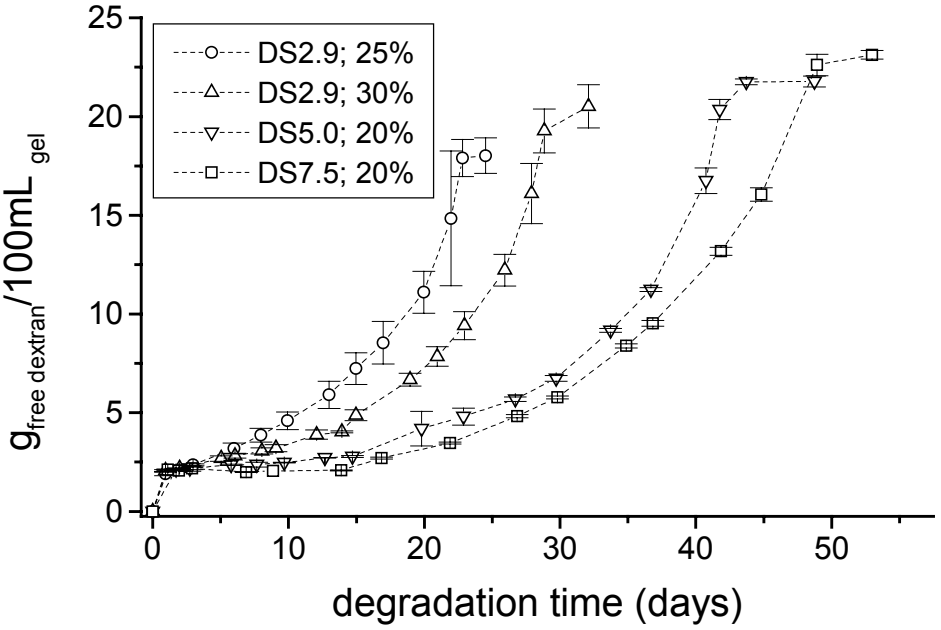

Figure 3. Variation of the amount of free dextran as a function of time in degrading dex-HEMA hydrogels. The data are the average of three independent measurements. The continuous lines were drawn arbitrarily through the data points.

Figure 3 shows the elastic modulus G' as a function of the degradation time for the dex-HEMA gels studied in Figure 2. This quantity exhibits a continuous decrease during the degradation process. The decrease of G' is significantly slower in gels having higher dex-HEMA concentration or higher DS. It was found previously that both the initial dex-HEMA concentration and DS affect the cross-link density of these networks[24]. An apparent rate constant can be determined from the decrease of G' by using the following relationship

$$G'(t)=G'(0)\exp(-k_{app}t) \qquad (3)$$

where G(0)' is the modulus of the undegraded gel and k_{app} is a constant. The values displayed in Table I indicate that the variation of the modulus in these highly swollen gels can be reasonably described by a pseudo-first order hydrolysis kinetics, and k_{app} decreases with increasing cross-link density. The latter result implies that degradation is slower in densely cross-linked gels.

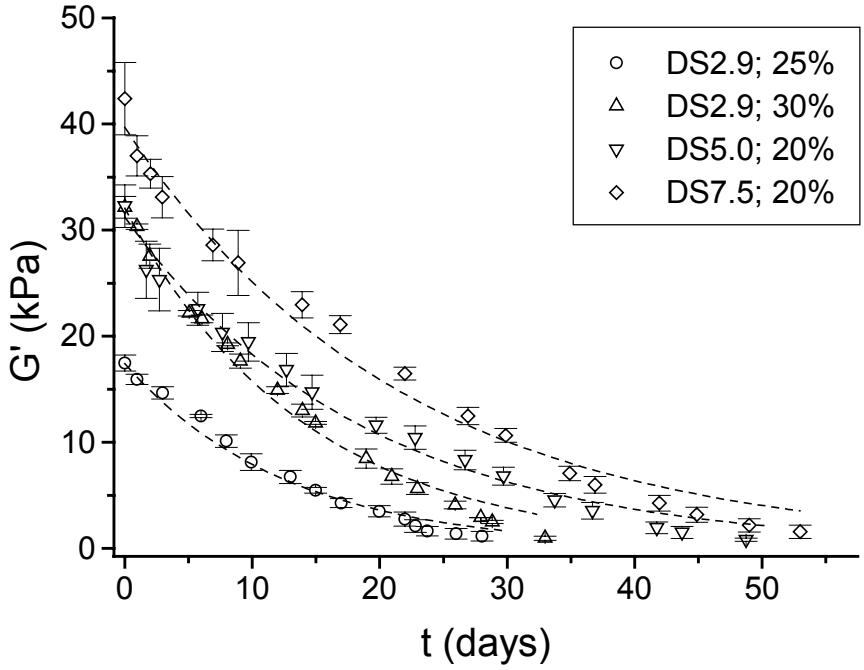

Figure 4. Variation of the elastic modulus as a function of time in degrading dex-HEMA hydrogels. The data are the average of three independent measurements. The dashed curves show the least squares fits to equation 3.

Tabel I: Fitting Parameters to Elastic Modulus (G') data (equation 3)

Gel Sample	G'(0)/kPa	k_{app}/day^{-1}
DS 2.9; 25%	17.5 +/- 0.7	0.079 +/- 0.003
DS 2.9; 30%	32.2 +/- 1.0	0.071 +/- 0.002
DS 5.0; 20%	32.3 +/- 2.0	0.056 +/- 0.002
DS 7.5; 20%	42.4 +/- 3.4	0.046 +/- 0.002

In order to reveal the effect of degradation on the thermodynamic properties we measured the swelling pressure Π_{sw} at different stages of degradation. The swelling pressure Π_{sw} of a non-ionic gel can be described as the sum of two terms[15]: an osmotic pressure Π_{osm} that expands the network, and an elastic pressure Π_{el} that acts against expansion

$$\Pi_{sw} = \Pi_{osm} - \Pi_{el} \qquad (4)$$

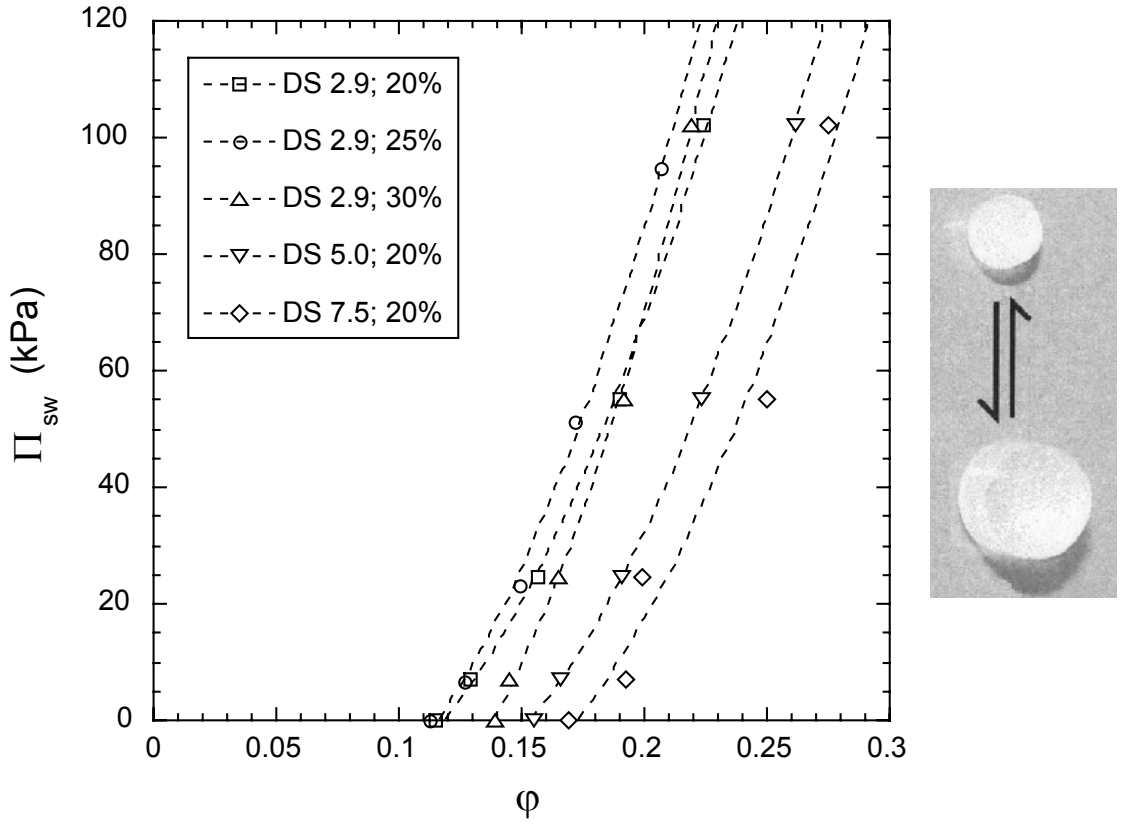

Figure 5. Plot of the swelling pressure vs polymer volume fraction for dex-HEMA gels before degradation. The legend shows the DS and the dex-HEMA concentration of the gels. Each measuring point is the mean value of measurements on three hydrogels, which were independently prepared. The dashed curves show the least-squares fits to equation 5. The image at the top shows a dex-HEMA gel shrunken in a PEG-solution while the image on the bottom shows the corresponding fully swollen dex-HEMA gel.

Figure 4 shows the variation of the swelling pressure as a function of the polymer volume fraction for different *undegraded* dex-HEMA hydrogels. The continuous curves in Figure 4 are the least squares fits of the swelling pressure data to the equation[16]:

$$\Pi_{sw} = A\,\varphi^{n} - A\,\varphi_e^{n-1/3}\,\varphi^{1/3} \qquad (5)$$

where A is a constant that depends on the particular polymer-solvent system, φ_e and φ are the volume fraction of the polymer in equilibrium with pure buffer and PEG solutions, respectively. For the exponent n scaling theory[25] predicts n=2.31 (good solvent condition) and n=3.0 (Θ-solvent condition). The values of A and n obtained from the fits to equation 5 are listed in Table II. As expected A depends on the chemical composition of the network. The value of n is close to that predicted for good solvent condition.

Tabel II: Parameters from the fit of the swelling pressure data to equation 5.

sample	degradation time (days)	Volume fraction[a]	A (kPa)	n	R[b]
DS 7.5; 20%	0	0.1150	4554	2.35	0.999
DS 5.0; 20%	0	0.1391	5767	2.33	0.999
DS 2.9; 20%	0	0.1547	3587	2.33	0.999
DS 2.9; 30%	0	0.1688	3231	2.36	0.981
DS 2.9; 25%	0	0.1128	5562	2.36	0.998
DS 2.9; 25%	3	0.0919	5563	2.34	0.997
DS 2.9; 25%	6	0.0745	5554	2.35	0.997
DS 2.9; 25%	10	0.0598	5487	2.34	0.997
DS 2.9; 25%	15	0.0493	5337	2.30	0.997
DS 2.9; 25%	27	<0.01	7920	2.33	0.993

[a] volume fraction at equilibrium swelling

[b] correlation coefficient

The effect of degradation on the swelling pressure was studied on the sample having the shortest degradation time (Dex-HEMA DS2.9; 25%). In Figure 5 is plotted the swelling pressure as a function of the polymer volume fraction measured at different stages of degradation (after 3, 6, 10, 15 and 27 days). The Π_{sw} *vs* φ curves are gradually shifted to the left as the gel degrades.

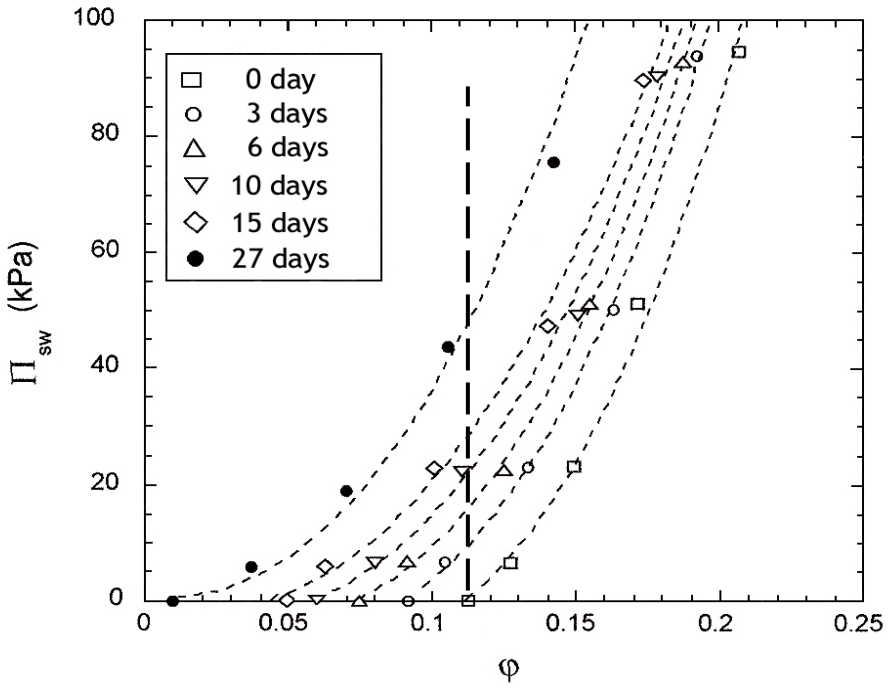

Figure 6. Swelling pressure of DS 2.9 dex-HEMA hydrogels deswollen in PEG-solutions. The dex-HEMA concentration at cross-linking was 25% (w/w). Before deswelling the hydrogels were degraded during 0, 3, 6, 10, 15 and 27 days, respectively. Each data point is the mean value of measurements on three hydrogels. The dashed curves through the data points are the least-squares fits to equation 5. The vertical line shows the increase in Π_{sw} at φ=0.112.

The parameters obtained from the fits of the swelling pressure curves of the dex-HEMA gels to equation 4 at different degradation times (Table II, Figure 6) indicate that neither *A* nor *n* varies notably in the first 15 days of degradation. It can also be seen that after 27 days (i.e., when the network is completely liquified) the value of *A* significantly increases.

We note that similar deviation between the values of *A* obtained for cross-linked and uncross-linked polymers has been reported for other polymer/solvent systems[26,27]. The difference in the A values can be attributed to the decreased degrees of freedom of the chains once cross-linked. This can be examined in terms of the interaction parameter χ. According to equation 6 [26]:

$$A = \frac{k_B T\left(C(1-2\chi)^{\frac{3}{4}}\right)}{k_s^3 a^3} \qquad (6)$$

wherein a is the length of a monomer, C is the polymer characteristic ratio, χ is the polymer-solvent interaction parameter, k_B is the Boltzmann constant and k_s is a constant of proportionality for a given polymer-solvent situation, the value of A decreases as χ increases. Indeed it has been established that χ for gels is greater than that for polymer solutions of equivalent polymer fraction.[27,28] This increase in the interaction parameter may be due to the reduction in configurational entropy imposed on the polymer-solvent interaction energies at the junctions and at the polymer chain interior[28]. However, a theory capable of describing these effects for prediction of A or χ has not yet been developed.

The dashed line in Figure 5 shows the situation that occurs when the gel is surrounded by a rigid semi-permeable membrane. During degradation Π_{sw} increases from 0 kPa (swelling pressure of the fully swollen undegraded gel) to 49 kPa (swelling pressure of the totally degraded dex-HEMA gel). The latter is the hydrostatic pressure required to maintain the initial concentration (φ=0.112) of the gel during the degradation process.

We note that in general the entrapped drug is expected contribute to the osmotic response. This effect depends on the drug load and the properties of the particular system (e.g., molecular weight of the drug and its interaction with the polymer matrix).

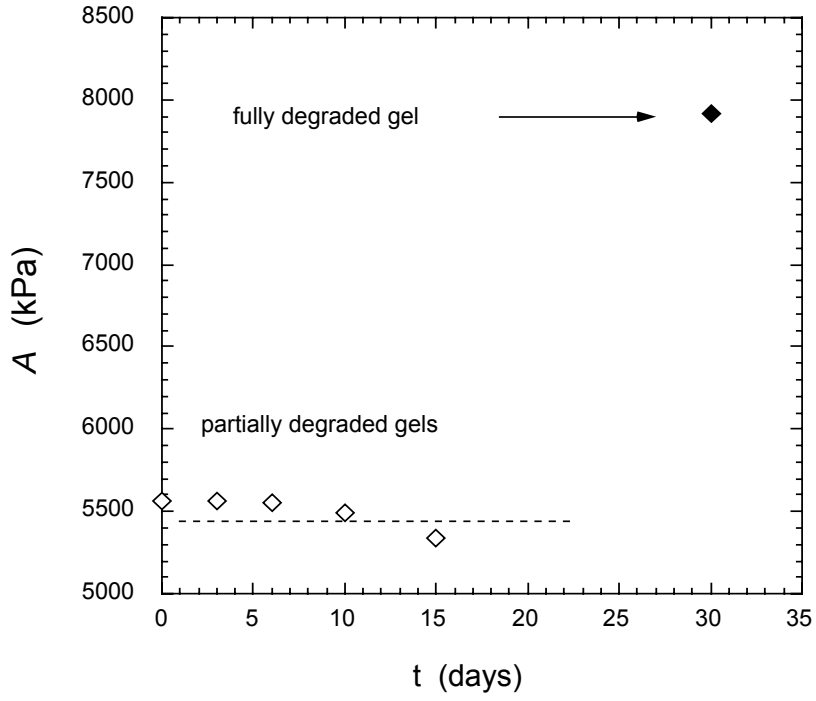

Figure 7. Variation of *A* as a function of degradation time t. Open symbols: partially degraded dex-HEMA gels; filled symbol: fully degraded dex-HEMA gel.

CONCLUSIONS

Osmotic deswelling measurements were performed on degrading dex-HEMA hydrogels to estimate the swelling pressure developed during network degradation. It is found that the degradation rate strongly depends on the initial dex-HEMA concentration and DS. The variation of the swelling pressure at each stage of degradation is satisfactorily described by the equation $\Pi_{sw} = A(\varphi^n - \varphi_{e,t}^{n-1/3}\varphi^{1/3})$, where $\varphi_{e,t}$ is the concentration of the fully swollen gel at degradation time t, and *A* and *n* are constants. In the earlier phase of the degradation process the swelling pressure gradually increases. This is caused by the decrease of the elastic pressure. Towards the end of the degradation process a pronounced increase in the swelling pressure is observed. This pressure change is accompanied by a sudden increase in the amount of dextran released from the gel.

In drug delivery systems the osmotic pressure can cause the rupture of the coat surrounding the hydrogel particle. Consequently, the knowledge of the variation of the swelling pressure during the degradation process is essential to design hydrogel systems that have a Π_{sw} profile tailored for pulsed delivery of drugs.

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3

Tailoring the swelling pressure on degrading dex-HEMA hydrogels

Abstract

Swelling pressure measurements were performed on degrading dextran hydroxyethylmethacrylate (dex-HEMA) hydrogels. In these networks the cross-links are hydrolysable carbonate ester bonds formed between methacrylate groups and dextran molecules. It is demonstrated that dex-HEMA gels made in the presence of known amount of free dextran chains exhibit osmotic properties similar to those of partially degraded dex-HEMA gels. The swelling pressure Π_{sw} of degrading dex-HEMA gels is controlled primarily by the cross-linked dex-HEMA polymer and the free dextran molecules, while the contribution of short poly-HEMA fragments (produced in the degradation process) is negligible. It is found that Π_{sw} only slightly changes during the first 15 days of degradation. Close to the end of the degradation process, however, a much faster increase in Π_{sw} is observed. The swelling pressure profile of these gels strongly depends on the concentration of the cross-linked dex-HEMA and its chemical composition (amount of HEMA groups per 100 glucose units).

INTRODUCTION

In the field of drug delivery there is a growing interest in biodegradable hydrogels.[1-5] To achieve *pulsed* drug delivery we propose a new concept based on the controlled variation of the swelling pressure of degrading hydrogels. We envision a delivery device that consists of a micron sized degradable gel particle surrounded by a water permeable membrane (Figure 1). As the gel gradually dissolves the swelling pressure (Π_{sw}) increases. When Π_{sw} exceeds the tensile strength of the surrounding membrane, the membrane ruptures and the drug is promptly released. In this 'degradation controlled exploding system' the release of the drug is governed by the degradation kinetics of the hydrogel particle. In this respect it is similar to the bursting capsules outlined in the patent of Baker,[6] and modeled by Kuethe et al.[7] The difference being that these devices rely on the degradation of a hydrogel whereas the system of Baker relies on small molecular weight compounds.

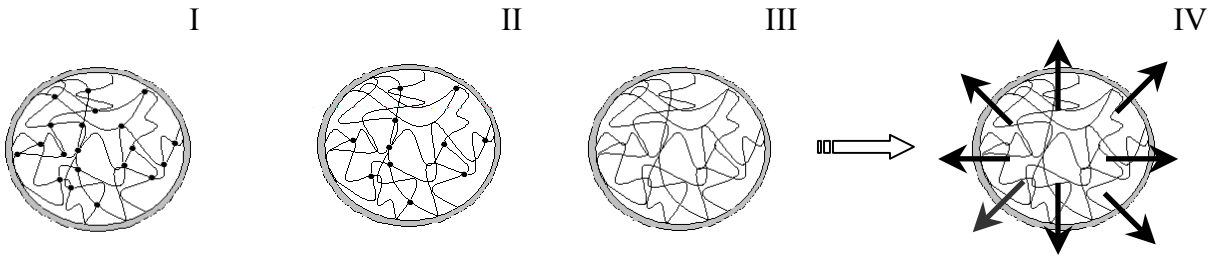

Figure 1. Shematic representation of a gel particle surrounded by a semipermeable membrane. **I.** Before degradation the polymer chains are connected into a three dimensional network by chemical cross-links (•). **II.** The gels described in this paper degrade as a result of hydrolysis of the cross-links. As degradation proceeds, the cross-link density decreases and free polymer chains are produced. **III.** At the end of the degradation process a polymer solution is present. IV. When Π_{sw} exceeds the tensile strength of the membrane, the membrane ruptures and drug molecules are released.

The swelling behavior of polymer gels has been investigated intensively.[8-11] Although many gel characteristics have been thoroughly studied, the variation of the swelling pressure during the degradation process has attracted only little attention. Recently we have reported swelling pressure measurements performed on degrading dextran hydroxyethylmethacrylate (dex-HEMA) hydrogels.[12] The cross-links in this system are hydrolyzable carbonate ester bonds formed between the methacrylate groups

and the dextran molecules. As the gel degrades free dextran chains as well as short poly-HEMA fragments are produced. We found that Π_{sw} after a weak initial growth exhibited a steep increase close to the end of the degradation process.

This knowledge of the variation of Π_{sw} during gel degradation is important to design pulsed delivery systems with tailored swelling pressure profile. Here we investigate the effect of different factors that influence the kinetics of degradation and Π_{sw}. The degree of substitution (DS, which is the amount of HEMA groups per 100 glucose units) and the initial concentration of dex-HEMA are varied. Furthermore, a comparison is made between Π_{sw} of degrading dex-HEMA gels and dex-HEMA/dextran gels. The latter system, containing controlled amounts of free (unattached) dextran chains, mimics partially degraded dex-HEMA gels since dextran is liberated as degradation proceeds. These investigations are expected to provide insight into the swelling properties of degradable dex-HEMA gels that may help to design 'membrane coated' gel systems for drug delivery after a certain lag time.

EXPERIMENTAL SECTION

Dex-HEMA preparation and characterization

The synthesis and characterization of hydroxyethylmethacrylated dextran was performed as described by Van Dijk-Wolthuis.[13] The number average molecular weight (M_n) of dextran (from Leuconostoc mesenteroides, Merck) and poly-HEMA were 19 000 g/mol and 1300 g/mol, respectively. (For the dextran M_w/M_n of 2.1 was found.) The degree of substitution (DS), defined as the number of HEMA groups per 100 glucose units, was determined by proton nuclear magnetic resonance spectroscopy in D_2O with a Gemini 300 spectrometer (Varian). Three batches of dex-HEMA with degree of substitution 2.9; 5.0 and 7.5, respectively, were prepared.

Preparation of dex-HEMA hydrogels and dex-HEMA/dextran hydrogels

Dex-HEMA hydrogels were prepared by radical polymerization of aqueous dex-HEMA solutions. The solution was prepared by dissolving dex-HEMA in phosphate buffer (PB: 10 mM Na_2HPO_4 adjusted with 1 N HCl to pH of 7.0). The polymerization reagents were N,N,N',N'-tetramethylene-ethylenediamine (TEMED; 20% v/v in deoxygenated PB, pH adjusted to 8.5 with HCl) and potassium persulphate (KPS; 50 mg/mL in deoxygenated PB). The gelation process was initiated by 50 µL TEMED solution and 90 µL KPS solution (per gram hydrogel). Gelation required approximately 1.5 hours. The extractable sol fraction of the dex-HEMA gels was less than 3%.

Dex-HEMA/dextran hydrogels were made using the same procedure in the presence of known amount of dextran. The solutions were prepared by dissolving dex-

HEMA and dextran in phosphate buffer. The dextran was the same as used in the dex-HEMA synthesis.

Hydrogel slabs used for the rheological measurements were made separately in cylindrical molds (diameter 2.3 cm, height 2 mm).

Swelling pressure measurements

Osmotic deswelling measurements were performed on degrading dex-HEMA gels using a method described by Horkay and Zrinyi.[9] Deswelling was achieved by enclosing the gels in a semipermeble membrane (Medicell dialysis bags, M_w cut-off between 12 000 and 14 000 g/mol). surrounded by aqueous poly(ethylene)glycol (PEG) solutions of known osmotic pressure. At equilibrium, the swelling pressure of the gel in the dialysis bag is equal to the osmotic pressure exerted by the PEG solution outside. After different degradation times dex-HEMA gel samples were equilibrated with PEG solutions at 4 °C. PEG (Merck, M_n of 20 000 g/mol) was dissolved in citrate buffer (9.44 g/L Na_2HPO_4; 10.3 g/L citric acid and 0.2 g/L NaN_3, pH of 4.4). It was verified that further degradation of the dex-HEMA gels did not occur during the measurements. Equilibrium swelling was attained within 7 days. The reversibility of the swelling process was checked.

The swelling pressure of gels prepared in the presence of free dextran chains was determined by a home-built 'swelling pressure osmometer'. This device consists of a callibrated transducer (Honeywell), a sample chamber (volume: 4.2 mL) and a buffer chamber (filled with 15 mL PB at pH of 7.0). The chambers are separated by a semi-permeable membrane (Medicell, M_w cut-off between 12 000 and 14 000 g/mol) supported by a porous Bekipor® frame which is further supported by a teflon perforated cylinder. The membrane is permeable to small molecules (water and ions) but impermeable to large dextran molecules. (The low molecular weight fraction of the dextran was removed by dialysis prior to the swelling pressure measurements.) The apparatus measures Π_{sw} up to 7 atmospheres. Π_{sw} measurements were performed on gels made in the sample chamber 12 hours after the preparation (i.e., before substantial degradation occurred). Measurements were made at 4 °C preventing hydrolysis of the dex-HEMA/dextran hydrogels. The experimental error of the swelling pressure measurements was found to be less than 5 %.

Rheological characterization of the hydrogels

Rheological measurements were performed by an AR1000-N controlled stress rheometer from TA-Instruments according to a method described in detail by Meyvis et al.[15] The elastic moduli of the gels were obtained from measurements made in oscillation mode at 1 Hz in the linear viscoelastic region by applying a constant strain of 0.5%.

RESULTS & DISCUSSION

In Figure 2A, a part of the osmotic deswelling profiles is presented for dex-HEMA hydrogels (DS2.9; 25%) degraded during 0, 3, 6, 10, 15 and 27 days, respectively. Considering the reversibility of the deswelling process, a dex-HEMA gel is able to develop a swelling pressure (y-axis in Figure 2A) equal to the osmotic pressure to which it was submitted. Prediction of the evolution of the swelling pressure of a coated degrading dex-HEMA hydrogel was obtained in two steps. First, dex-HEMA hydrogels were degraded, resulting in a swelling as a function of degradation time. This is reflected in the Π_{sw} vs φ plots which gradually shift to the left as the gel degrades. After different degradation times, the degradation was inhibited and the hydrogels were deswollen with PEG-solutions to the initial polymer volume fraction of the non-degraded hydrogel. The values on the vertical line in Figure 2A, represent the pressure necessary to keep the polymer volume fraction of a coated dex-HEMA hydrogels constant during degradation. In order to compare the swelling pressures of the dex-HEMA/dextran gels (measured in the swelling pressure meter) with the swelling pressures of the dex-HEMA hydrogels (measured with osmotic deswelling), the initial polymer volume fraction is 0.151 instead of 0.113, representing the relaxed or non-swollen state in stead of the swollen state of the gels.

In Figure 2B is presented the variation of Π_{sw} of a dex-HEMA gel obtained from swelling pressure measurements as a function of the degradation time t. The figure also shows the osmotic ($\Pi_{osm,\ t}$) and elastic ($\Pi_{el,\ t}$) components of the swelling pressure calculated by the following equation:[9; 16; 17]

$$\Pi_{sw,t} = \Pi_{osm,t} - \Pi_{el,t} = A\varphi^{n} - A\varphi_{e}^{n-\frac{1}{3}}\varphi^{\frac{1}{3}} \qquad (1)$$

where φ and φ_e is the polymer volume fraction of the cross-linked dex-HEMA in the non-degraded gel (with $\varphi = 0.151 \pm 0.006$) and of the cross-linked dex-HEMA in the fully swollen gel after degradation time t, respectively, *A* is a constant and n is a scaling exponent that depends on the thermodynamic quality of the solvent. In the analysis of the swelling pressure data *A* and n were iteratively adjusted to minimize the variation of the Π_{sw} for each set of data points. The resulting values of *A* and n are displayed in Table II of chapter 2.

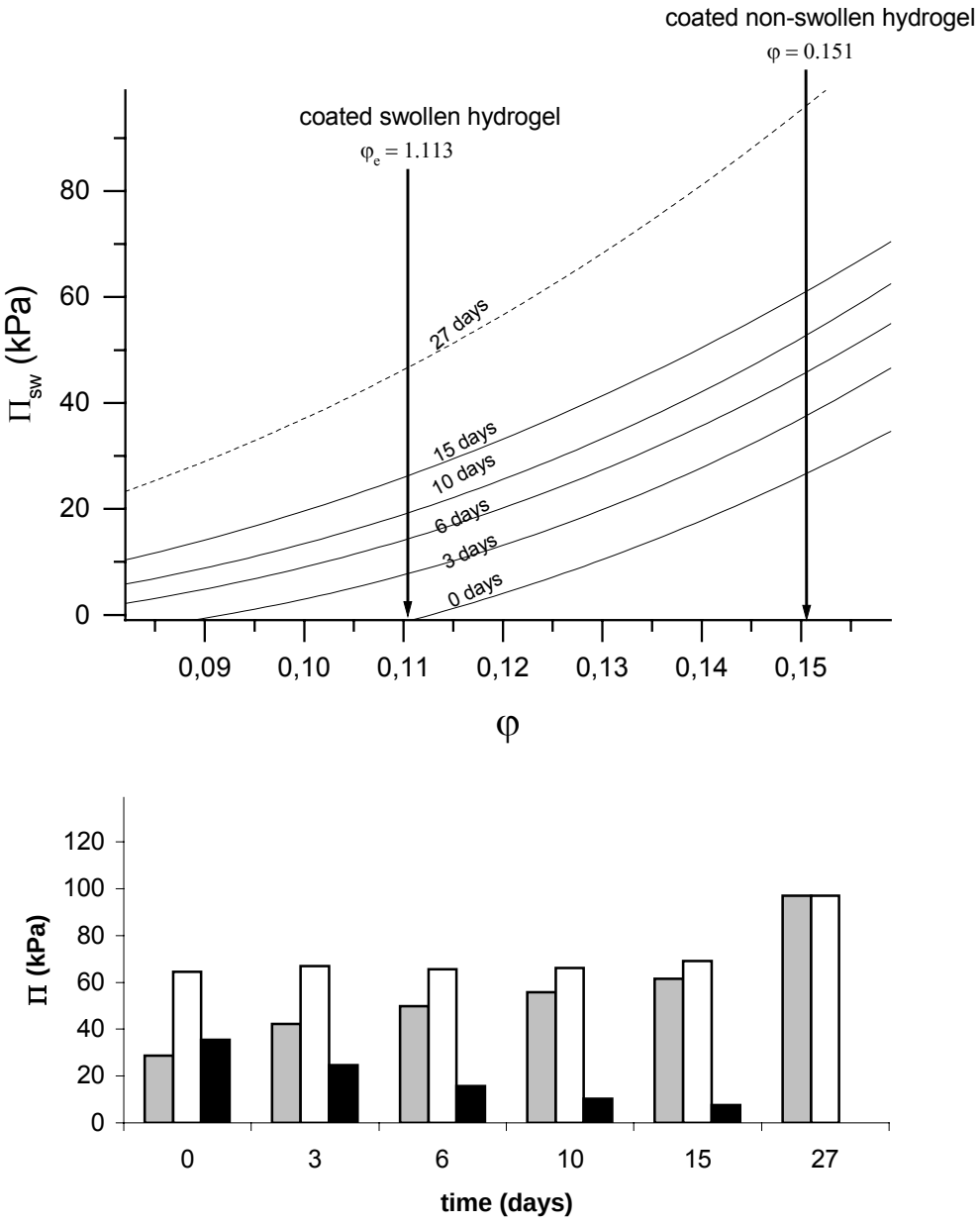

Figure 2A. A part of the swelling pressure curve fittings (see Figure 6 of Chapter 2) of the degrading dex-HEMA (DS of 2.9) hydrogels is represented. The dex-HEMA concentration at cross-linking was 25% (w/w). The vertical lines show the increase in swelling pressure at φ_e = 0.113 and at φ = 0.151, respectively. **B.** Osmotic pressure (open bars), elastic pressure (black bars) and swelling pressure (gray bars) of dex-HEMA hydrogels (DS2.9;25%) during degradation. The swelling pressure was obtained from osmotic deswelling measurements with a constant φ = 0.151 (see Figure 2A), while the elastic and osmotic pressures were calculated from the measured swelling pressure by equation 1.

Figure 2B shows that Π_{el} decreases and Π_{sw} increases as the gel degrades (i.e., the carbonate ester cross-links are hydrolyzed). In the first 15 days of the degradation process no appreciable change in Π_{osm} can be observed. (After 15 days this dex-HEMA gel became too weak to manipulate and to make swelling pressure measurements.) At the end of the degradation, when the dex-HEMA gel turned into a polymer solution, osmotic pressure measurements were performed. This 'gel-sol' transition is accompanied by a sudden increase in the osmotic pressure indicating significant differences between the osmotic properties of the cross-linked polymer and the corresponding polymer solution. Similar observations have been reported for other polymer/solvent systems.[18; 19]

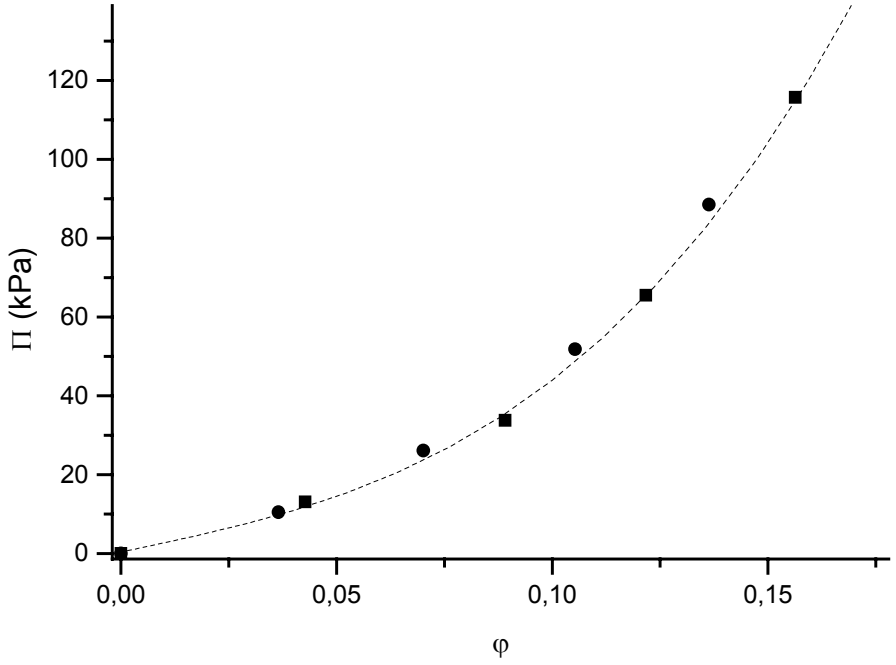

Figure 3. Osmotic pressure of totally degraded dex-HEMA gels (DS 2.9; ●) and dextran solutions (■) as a function of the concentration.

In order to estimate the osmotic contribution of the degradation products (low molecular weight poly-HEMA fragments and free dextran chains) we compared the osmotic pressure of a totally degraded dex-HEMA gel and that of a dextran solution (Figure 3). The good agreement between the numerical values indicates that poly-HEMA fragments do not make a significant contribution to the swelling pressure of degrading dex-HEMA gels. This finding is consistent with the expectation that low molecular weight degradation products (from GPC measurements M_W(poly-HEMA) < 1300 g/mol) diffuse through the semipermeable membrane and leave the gel matrix.

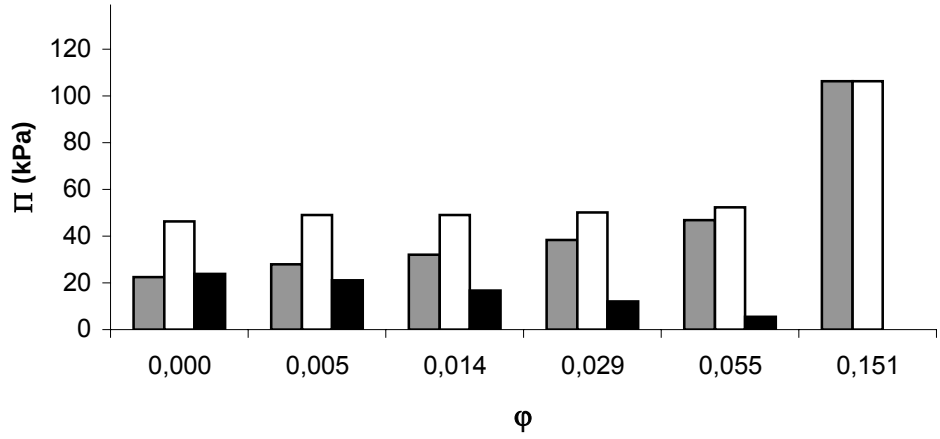

Figure 4. Osmotic pressure (open bars), elastic pressure (black bars) and swelling pressure (gray bars) of dex-HEMA/dextran hydrogels containing increasing amounts of free dextran chains. The DS of the dex-HEMA is 2.9. The total polymer volume fraction (dex-HEMA + free dextran) is 0.151 (± 0.006) for all the gels. The values on the x-axis indicate the volume fractions of *free* dextran in dex-HEMA/dextran gels.

Figure 4 shows the dependence of Π_{sw}, Π_{el} and Π_{osm} on the volume fraction of free dextran chains $\varphi_{dextran}$ in dex-HEMA gels prepared in the presence of known amounts of dextran (dex-HEMA/dextran gel). Π_{sw} was measured by the swelling pressure osmometer, Π_{el} was estimated from elastic modulus measurements using the relationship $\Pi_{el} = G'$, where G' is the shear modulus, [20; 21] and Π_{osm} was calculated from Π_{sw} and Π_{el} by equation 1. The dextran concentration on the x-axis corresponds to $\varphi_{dextran}$ in degrading dex-HEMA gels after degradation times 0, 3, 6, 10, 15 and 27 days, respectively. The amount of free dextran was calculated from dextran release data of dex-HEMA gels measured by GPC (see ref.[12]). Figure 4 shows that Π_{el} decreases, Π_{sw} increases, and Π_{osm} only weakly varies as the volume fraction of free dextran increases. Upon approaching the gel-sol transition, however, Π_{osm} rapidly increases. Clearly, the behavior of these dex-HEMA/dextran gels is similar to that of partially degraded dex-HEMA gels.

In Figure 5 the quantity Π_{sw} + G' is plotted as a function of $\varphi_{dextran}$ for dex-HEMA/dextran gels. These gels differ in the degree of substitution (2.9<DS<7.5) and the total polymer concentration (cross-linked dex-HEMA + uncross-linked dextran). In the Figure is also shown the concentration dependence of the osmotic pressure of the uncross-linked dextran solution. At low dextran concentration ($\varphi_{dextran} < 0.1$) Π_{sw} + G' is primarily governed by the polymer concentration. As $\varphi_{dextran}$ increases Π_{sw} + G' exhibits an increase and gradually approaches the osmotic pressure of the dextran solution.

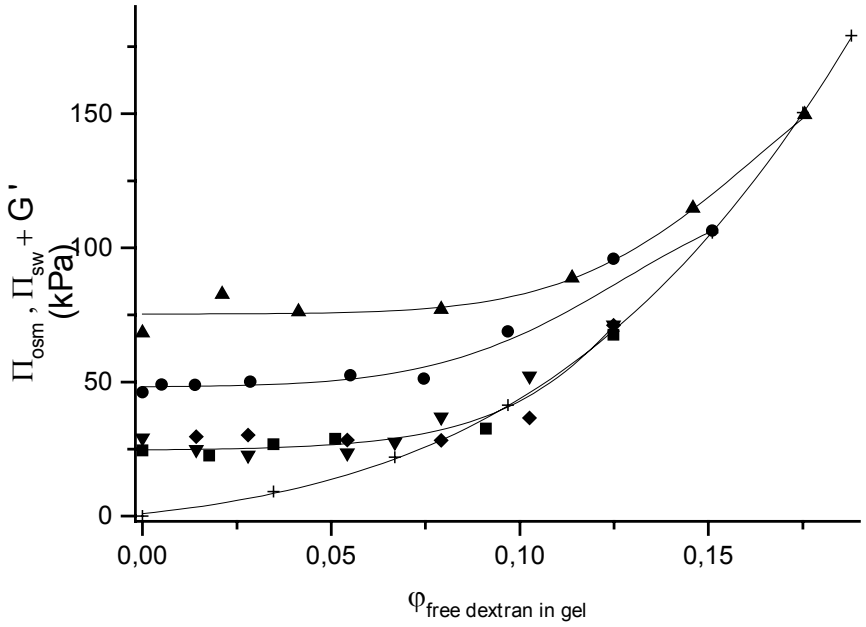

Figure 5. Π_{sw} + G' for dex-HEMA/dextran hydrogels containing increasing amounts of free dextran. The DS and the total polymer concentration (dex-HEMA + dextran) of these gels are as follows: DS2.9; 25% (●); DS2.9; 30% (▲) ; DS3.5 ;20% (♦); DS5.0; 20% (▼); DS7.5; 20% (■). The values on the x-axis indicate the volume fractions of the free dextran in dex-HEMA/dextran gels. The continuous line shows the osmotic pressure of the uncross-linked dextran solution (+).

To develop a degradation controlled exploding system as a platform for pulsed drug delivery, it is desirable to design hydrogels that possess different degradation kinetics.[22; 23] In Figure 6 are presented swelling pressure versus degradation time plots obtained from swelling pressure measurements of dex-HEMA/dextran gels in conjunction with dextran release data of degrading dex-HEMA gels (see Figure 2 Chapter 2). As swelling pressure measurements are performed on hydrogels in the non-swollen state

(relaxed state), the data of the release experiments were also recalculated towards gels in their relaxed state. The composition of these systems corresponds to that of various dex-HEMA hydrogels at different stages of degradation. In Figures 6 A and B the influence of dex-HEMA concentration is depicted. The initial value of the elastic modulus, the height of the 'plateau' region of the osmotic pressure plot, and the degradation time increase with increasing dex-HEMA concentration. Figures 6 C and D illustrate the effect of DS. Increasing DS increases the amount of possible cross-links. Generally, increasing the cross-link density (due to increasing either DS or dex-HEMA concentration) lengthens the degradation time and, consequently, the time required to attain a certain swelling pressure.

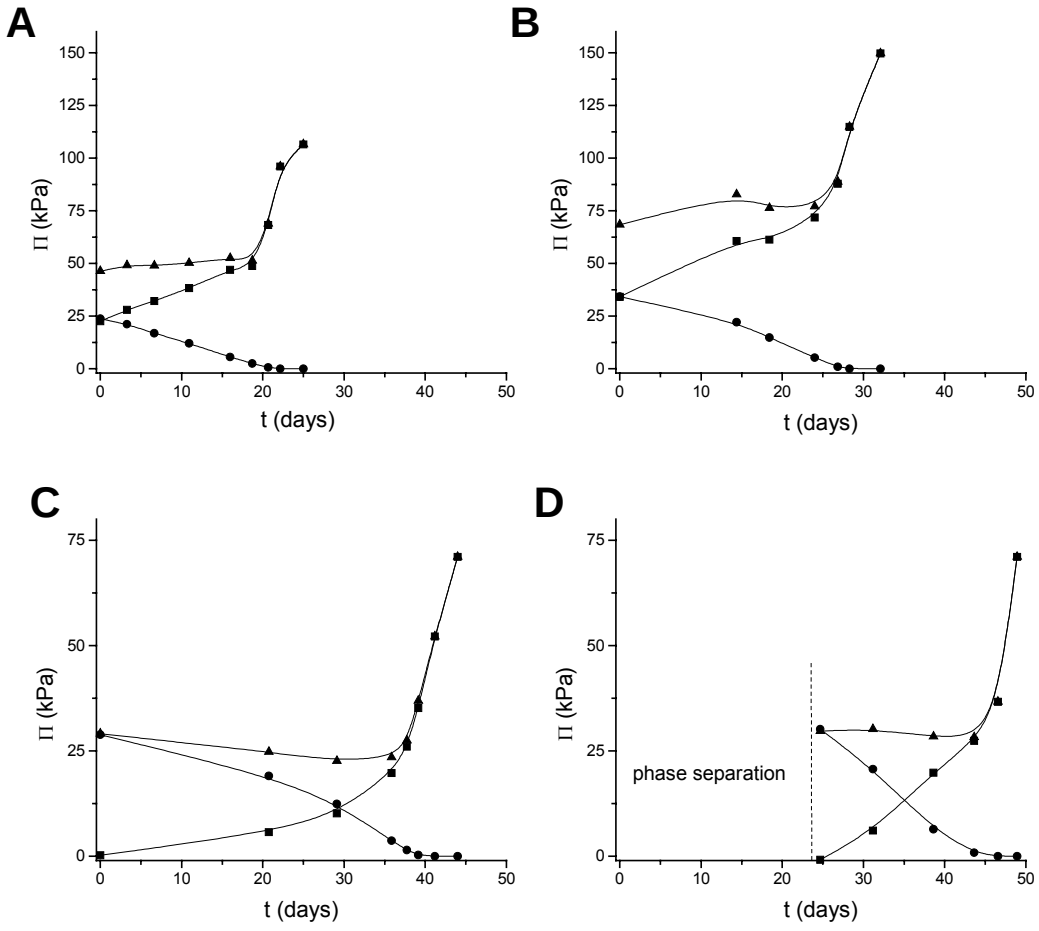

Figure 6(A-D). Variation of the elastic (●), osmotic (▲) and swelling pressure (■) of different dex-HEMA hydrogels as a function of the degradation time. Symbols: **(A)** DS2.9; 25% **(B)** DS2.9; 30% **(C)** DS5.0; 20% **(D)** DS7.5; 20%. Note that at low dextran concentration the dex-HEMA/dextran gel (DS7.5; 20%) exhibits phase separation, therefore, in this concentration range osmotic measurements were not be made.

CONCLUSIONS

A systematic study was performed to gain insight into the effect of different factors (concentration of free dextran chains, degree of substitution, total polymer concentration) on the swelling pressure of degrading dex-HEMA hydrogels. In these networks the cross-links are hydrolyzable carbonate ester bonds formed between methacrylate groups and dextran molecules. It is demonstrated that dex-HEMA/dextran gels made in the presence of known amount of free dextran chains exhibit swelling pressure similar to those of partially degraded dex-HEMA gels. It is found that Π_{sw} only slightly increases during the first 15 days of the degradation process. We showed that in this period Π_{osm} of the gels did not change significantly. Close to the gel-sol transition, however, Π_{sw} rapidly increases. The swelling pressure profile of these gels, particularly the variation of the swelling pressure in the final phase of gel degradation, strongly depends on the concentration of the cross-linked dex-HEMA and the chemical composition of the network chains.

ACKNOWLEDGEMENTS

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4

Development of an osmometer for swelling pressure measurement of (degrading) hydrogels

Abstract

A new type of device is proposed that uses the sensitivity and reliability of a pressure transducer to measure the swelling pressure of complex systems as biodegradable hydrogels. In the proposed device, degradation induced changes in the swelling pressure of pharmaceutical hydrogels are measured by confining the hydrogel in an enclosure between a rigid semi-permeable membrane and the diaphragm of a pressure transducer. The concept of the osmometer is designed to keep the volume of the sample chamber constant during the measurements and hence to avoid swelling of the hydrogel. The sensor response time of the osmometer is checked. Validation of this device is performed using dextran and poly(ethylene)glycol solutions of known osmotic pressure. Values of the swelling pressure measured in the osmometer agree with literature values of the same solutions. The osmometer has the potential to detect the effect of degradation on the thermodynamic properties as swelling pressure of pharmaceutical hydrogels. Knowledge of the swelling pressure and especially its evolution during degradation is extremely important for the design of new pulsatile drug delivery systems combining degrading hydrogels and osmotic bursting of a water-permeable coat.

INTRODUCTION

In this work, knowledge of the evolution of the constant changing osmotic properties of a degrading hydrogel is essential.[1-3] Not only the maximal reachable pressure value but also the evolution of the pressure profile, governed by the degradation kinetics of the hydrogel, will determine the time of osmotic bursting of the proposed exploding membrane surrounded microgels. The concept of performing pressure measurements in order to obtain hydrogel osmotic properties goes back at least 30 years to van de Kraats.[4] In more recent literature hydrogel osmotic properties were measured either by mechanical compression or by osmotic deswelling.[5-7] However, the use of these techniques for evaluation of biodegradable hydrogels with constant changing properties as a function of time is limited. In 2002, Han et al. reported on a constant-volume hydrogel osmometer as new device concept for miniature biosensors.[8] The necessity to make the gels outside the device (M-Biotech) and especially the large mesh size of the rigid semi-permeable membrane makes the concept not useful for our application.

Figure 1A shows a conventional approach for biodegradable hydrogels. A biodegradable hydrogel is a cross-linked network that gradually liquefies in response to degradation. Degradation causes network destruction resulting in the increase of the swelling ratio and the release of degradation products as a function of time. However, for the determination of the osmotic properties of these biodegradable hydrogels, changes in network structure as well as the presence of degradation products will play a major role and will contribute to the swelling pressure measured. In Figure 1B, the same hydrogel is confined to a volume that is essentially constant, between a rigid porous membrane and a high performance pressure transducer. The membrane is only permeable to water and not permeable for the degradation products of the hydrogel. In this way, degradation products are prevented to leave the confined hydrogel. When degradation occurs, hydrogel properties change, water will diffuse into the hydrogel until the favorable energy of mixing is balanced by the increase in mechanical pressure within the enclosure. So, when a gel is constrained and can not reach its equilibrium swelling, pressure Π is build-up in the gel phase to compensate for the 'gab' in the chemical potential. Based upon this concept, a device was designed that should allow constant monitoring of the osmotic properties of biodegradable hydrogels. With the constant volume concept, the pressure and thermodynamic behavior of the hydrogel is very similar to its behavior in a drug delivery device consisting of a hydrogel core surrounded by a rigid semi-permeable membrane.

In this chapter the *'osmometer or swelling pressure device'* is presented. The sensor response time is evaluated. Validation is performed with PEG and dextran solutions with known osmotic pressure. The swelling pressure of a dex-MA (DS3.1 ; 25%) hydrogel obtained with the osmometer and with the osmotic deswelling method, respectively, are compared. The applicability of the device as constant monitor of changing osmotic properties of biodegradable hydrogels is illustrated for an enzymatically degrading dex-

MA hydrogel. The influence of enzyme concentration on the pressure profile is also shown.

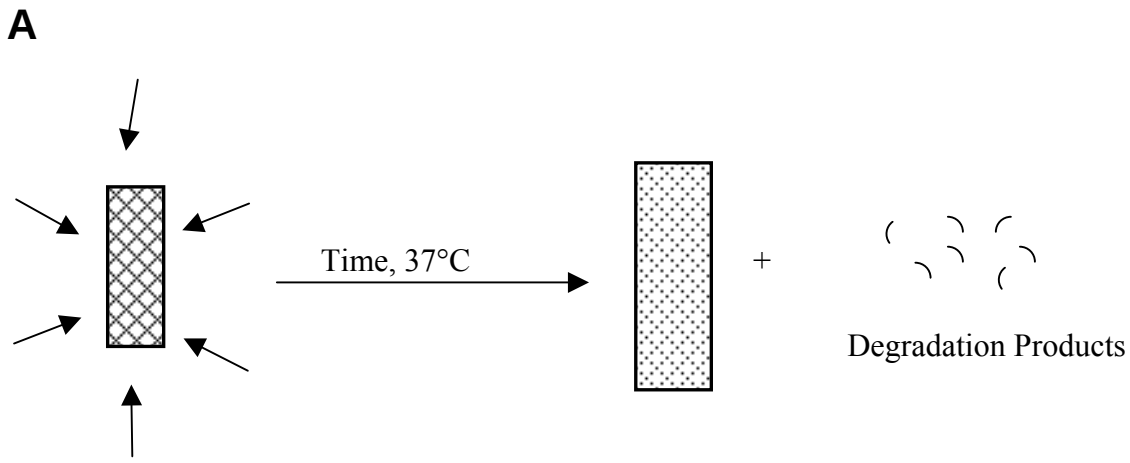

Figure 1A. Swelling and liberation of degradation products from the unconfined biodegradable hydrogel. **B.** Biodegradable hydrogel at fixed volume, confined between the semi-permeable membrane and the diaphragm of the pressure transducer.

DESIGN OF THE OSMOMETER

A

Figure 2A. Photograph of the osmometer or swelling pressure device.

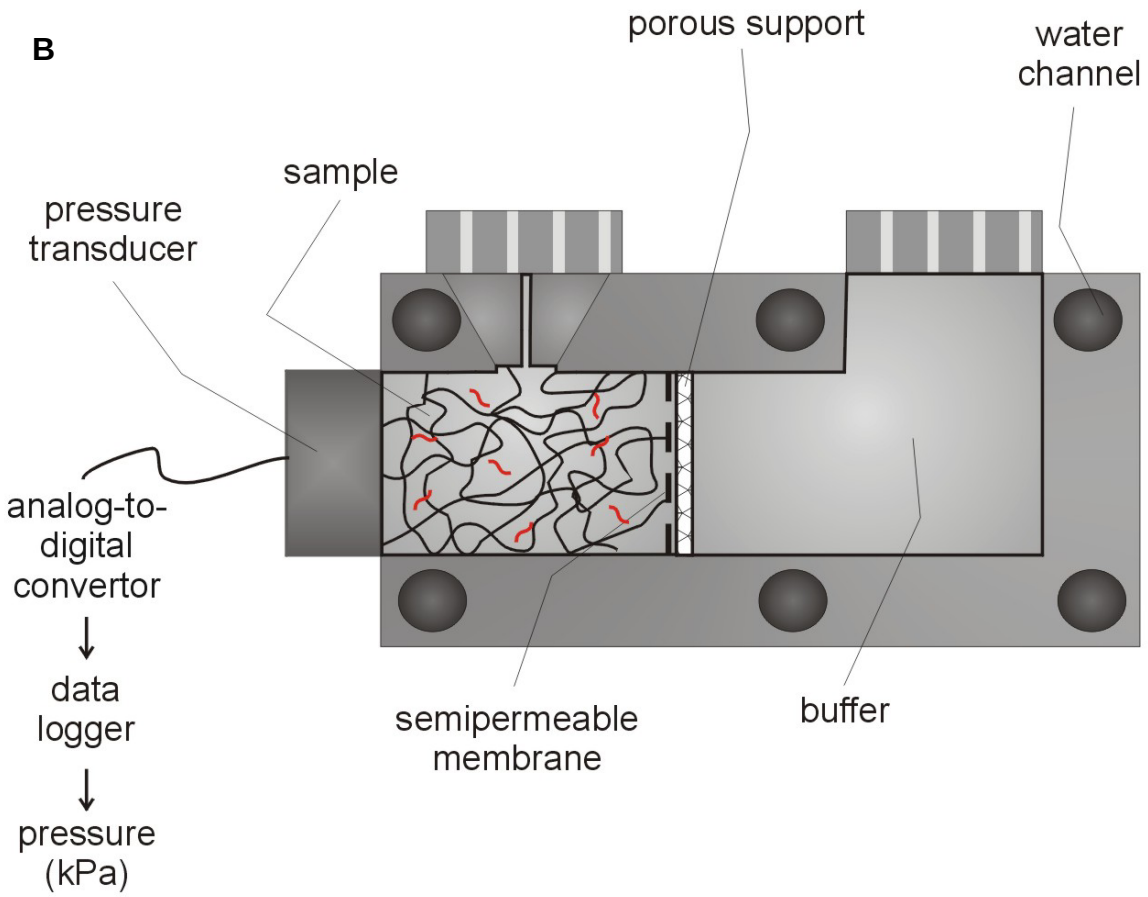

Figure 2B. Schematic representation of the home-made osmometer. The hydrogel is moulded in the sample chamber resulting in perfect contact between pressure transducer and hydrogel and hydrogel and the semi-permeable membrane, which is supported on a porous wire frame. The water channels of the device are connected to a warm water bath allowing thermostatisation. The stainless steel pressure transducer is calibrated up to 690 kPa.

The home-made osmometer (which has also been called the *swelling pressure device* throughout this work) is illustrated in Figure 2. It is essentially a membrane osmometer. Compared with commercial membrane osmometers which are able to withstand pressures up to 0.01 atmospheres, the proposed osmometer is able to withstand much higher pressures (i.e. up to 7 atmospheres). Except for the constant volume hydrogel osmometer which has been proposed recently[8], the use of commercial membrane osmometers is restricted to the pressure measurement of solutions (e.g. protein solutions with a molecular weight higher than 10 000 g/mol). To our knowledge, pressure measurement of hydrogels and more specifically measurement of the pressure changes of degrading hydrogels has not been reported.

The *osmometer* or *swelling pressure device* is built in stainless steel (18/10) and consists of a calibrated transducer (Stainless Steel Pressure Transducer Model AB/HP, Honeywell, 0-7 atmospheres), a sample chamber (4.2 mL) and a buffer chamber (15 mL). Both chambers are separated by a semi-permeable membrane supported by a porous filter frame (48 mesh, Bekipor®ST, Bekaert Fibre Technology) which is further supported by a teflon perforated cylinder.

Based upon the molecular weight of the degradation products (formed during hydrogel break-down) a certain molecular weight cut-off (MWCO) is chosen for the semi-permeable membrane. The lower the MWCO the slower the diffusion and the larger the equilibrium time which results in a longer sensor response time. Membranes with MWCO ranging between 100-14 000 g/mol are used. In order to keep the hydrogel confined to a constant volume the sample chamber is sealed. Evaporation of the buffer solution is prevented by sealing the buffer chamber. As the pressure is highly temperature dependent, the device is provided with water channels which are connected to a warm water bath (Hetofrig) to allow thermostatisation. During pressure measurements data are logged on a voltmeter (Consort, 0-100 mV). Pressures are calculated based upon the linear relationship: (Π (kPa) = 6,4898 . U (mV)), which was provided with the calibrated sensor of Honneywell.

EXPERIMENTAL SECTION

Preparation of polymer solutions

Poly(ethylene)glycol and dextran solutions were prepared by dissolving the corresponding polymers, into phosphate buffer (PB:10 mM, Na_2HPO_4, 0.02 % sodiumazide, adjusted with 1 M hydrochloric acid to pH of 7.0). Poly(ethylene)glycol (PEG, Merck) with a molecular weight M_n of 20 000 g/mol was used. Dextran (Fluka, from *Leuconostoc ssp*.) with a molecular weight M_n of 19 000 g/mol was used. The concentration of the polymer solutions ranged between 0 to 30% (w/v). About 5 mL of each sample was immersed in the osmometer. The pressure was measured at 25°C.

Preparation of dex-MA/dextranase hydrogels

Dex-MA/dextranase gels were made by radical polymerization of aqueous dex-MA/dextranase solutions[9,10]. The DS of Dex-MA used in this study was 3.1 or 4.0. The solutions were prepared by dissolving dex-MA in a phosphate buffer (10 mM, pH of 7.0). Dextranase, the enzyme (D-1508 Sigma; diluted to 10 U/mL in PB, one unit liberates 1 µmol of isomaltose per minute at pH of 6.0 and 37°C) was added to the dex-MA solution (cooled to 4°C) prior to the addition of the gelation reagents. The polymerization reagents were N,N,N',N'-tetramethyleneethylenediamine (TEMED: 20% v/v in deoxygenated PB,

pH adjusted to pH of 7.0 with hydrohloric acid) and potassium persulphate (KPS: 50 mg/mL in deoxygenated PB). 50 µL of TEMED solution was added to 1 g of polymer solution. After homogenization, 90 µL of KPS was subsequently added to the system to initiate gelation. The gelating mixture was immersed directly in the osmometer (cooled to 4°C). On average a complete gelation took 90 minutes at 4°C. Throughout this work the concentration of the hydrogel was always 20-25% (w/w) and refers to the concentration at cross-linking.

Swelling pressure measurement

Osmometer

Π_{sw} of the dex-MA/dextranase hydrogels was measured in the 'home-made osmometer'. A Spectra Por® cellulose ester membrane with molecular weight cut-off of 100 g/mol was used. The membrane is permeable to water but impermeable to the low molecular weight degradation products of the dex-MA hydrogels used in this study. Hydrogel gelation occurred at 4°C. After 12 hours of equilibration with the buffer, hydrogel degradation is allowed at 37°C. The buffer chamber is filled with PB (10mM, pH of 7.0) containing equimolar concentrations of KPS, TEMED and dextranase as used in the hydrogels. This is necessary because they are to large to migrate through the membrane and would contribute to the measured swelling pressure.

For measurement of the osmotic pressure of the dextran and PEG-solutions, an equilibration time of 4 hours was respected. As semi-permeable membrane a Medicell dialysis bag with MWCO of 12-14000 g/mol was used.

Osmotic deswelling

Osmotic deswelling measurements were performed on degrading dex-MA gels using a method described by Horkay and Zrinyi.[7] (see also Figure 2 of Chapter 2) Deswelling was achieved by enclosing the gels in a semi-permeable membrane (Medicell dialysis bags, MWCO between 12 000 and 14 000 g/mol) surrounded by aqueous poly(ethylene)glycol (PEG) solutions of known osmotic pressure. Equilibrium swelling (at 4°C) was attained within 7 days.

RESULTS & DISCUSSION

Evaluation of the sensor response time

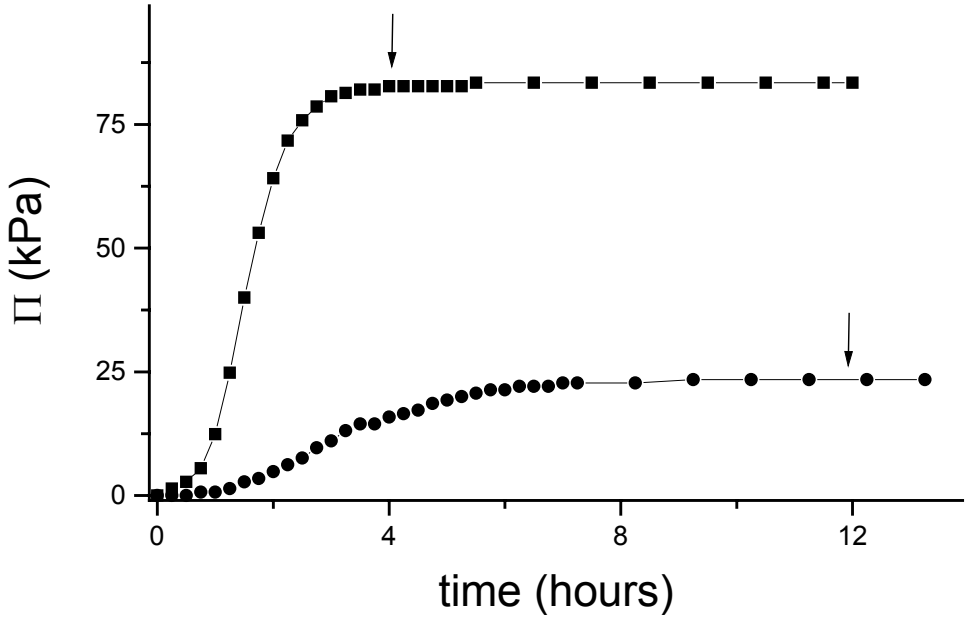

Figure 3. Evaluation of the sensor response time by pressure measurement of a dextran solution (20% (w/w)) and of a dex-MA hydrogel (DS3.1; 20%) as a function of equilibration time.

An ideal pressure device should be able to measure the osmotic or swelling pressure of a sample immediately when the sample comes in contact with the buffer solution. Therefore the response time of the sensor of the osmometer is checked for a dextran (20% (w/w)) solution and for a dex-MA hydrogel (DS3.1; 20% (w/w)). In both cases, a semi-permeable membrane with MWCO of 12-14 000 g/mol is used. As shown in Figure 3, a constant pressure value is reached after 3 hours and 6 hours equilibration for a dextran solution and a dex-MA hydrogel, respectively. Therefore, in this work, a sensor response time of 4 and 12 hours is used for polymer solutions and hydrogels, respectively. In this way equilibrium osmotic and swelling pressure is assured before any swelling pressure measurement is performed. Han et al. published recently a miniature biosensor which is basically a similar system.[8] For 0.3 mm hydrogels a response time of 20 minutes was obtained. Consequently, the long sensor response time of our device can be reduced substantially by using a miniaturised sample chamber reducing the thickness of the

hydrogel. As a very small deviation in the sensor diaphragm is necessary before detection of a signal, reduction of the sensor response time can be explained by the reduction of the diffusion time of water in the sample chamber with reduced lenght. As the diffusion of water in a polymer solution is faster compared to the diffusion of water in a polymer network the longer response time of the dex-MA gel towards the dextran solution (see Figure 3) is also explained.

Measurement of the osmotic pressure of polymer solutions

In Figure 4, the osmotic pressure of dextran-solutions and PEG-solutions are plotted as a function of polymer concentration. The reproducibility of the Π_{sw}-measurements was found better than ± 2%. As also shown in Figure 4, for both polymer solutions, dextran and PEG, similar pressure values were obtained as reported by Edmond and Ogston in 1968.[11] Despite the similarity in molecular weight of both polymers used, higher pressure versus concentration values are obtained for PEG compared to dextran. This is explained by differences in polymer-solvent and polymer-polymer interactions.

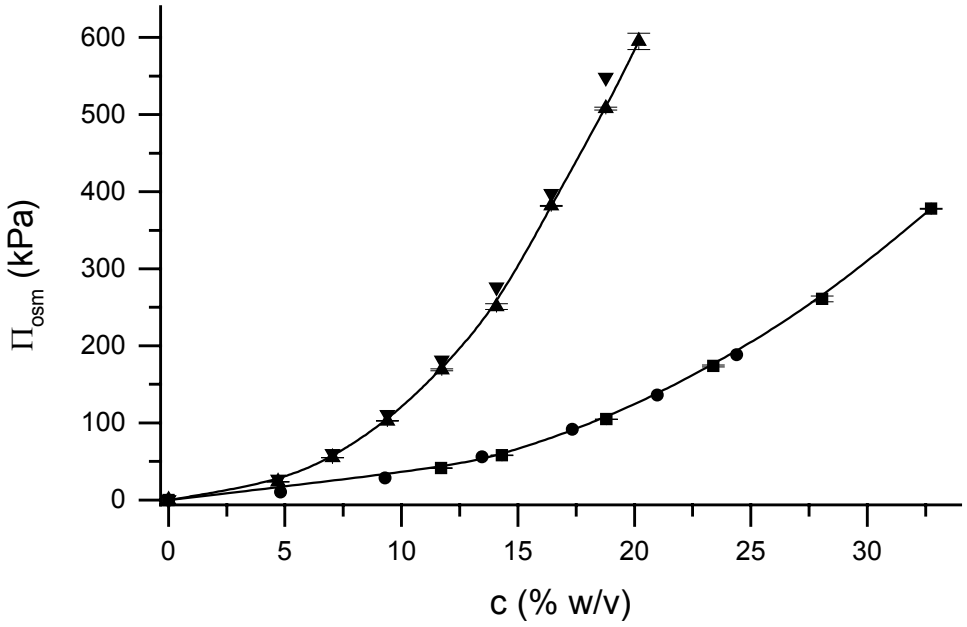

Figure 4. Pressure measurement of dextran (■) and poly(ethylene)glycol (▲) solutions in the home-made osmometer in comparison with literature data of similar solutions, dextran (●) and PEG (▼), respectively).[11] Measurements were performed at 25°C, after 4 hours of equilibration with the buffer solution. The data are an average of at least three repeats.

Measurement of the osmotic pressure of hydrogels

In Figure 5, a comparison was made between the swelling pressure of a dex-MA hydrogel (DS3.1; 25%) obtained by osmotic deswelling and obtained by the osmometer, respectively. It takes about 69 kPa of additional osmotic stress (exerted by the PEG-solution) to keep the volume of the dex-MA hydrogel fixed. If the sensor is accurate, it should take about 69 kPa of additional mechanical pressure to keep the hydrogel volume fixed. From Figure 5, the value measured with the sensor of the osmometer is 64 ± 10 kPa, including hydrogel batch-to-batch variations. We consider both methods for swelling pressure measurement of hydrogels to be in acceptable agreement.

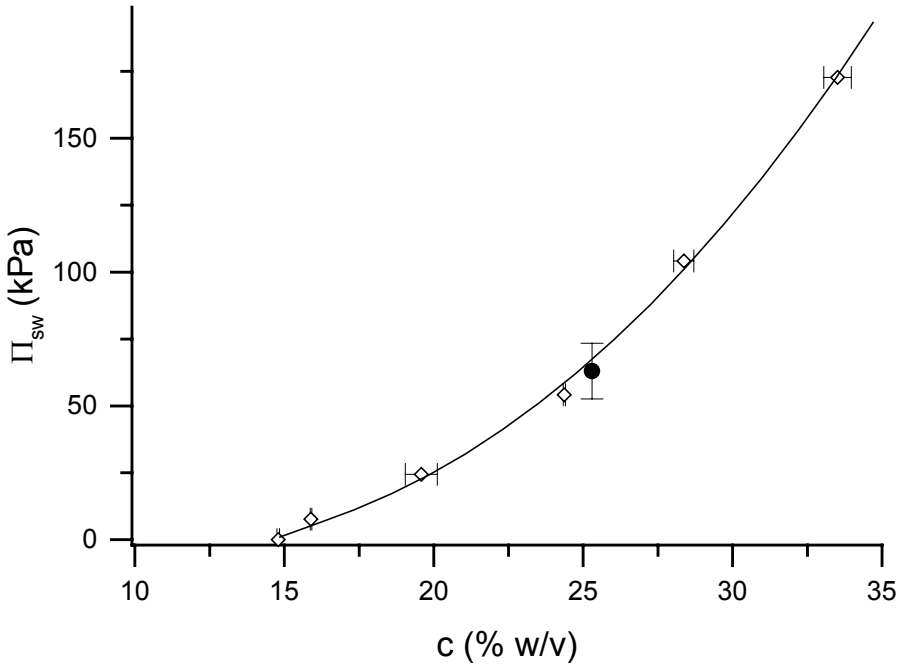

Figure 5. Swelling pressure of a dex-MA hydrogel (DS 3.1; 25%) as measured by osmotic deswelling (open symbols) and by the constant volume hydrogel osmometer (closed symbol), respectively. The data are an average of at least three repeats.

Measurement of the osmotic pressure of degrading hydrogels

In order to evaluate the osmometer as possible tool for monitoring constant changes in osmotic properties, an enzymatic degradable hydrogel system is chosen. Degradation of Dex-MA by the entrapped endoenzym dextranase results in the formation of small molecular weight degradation products. Therefore, a semi-permeable membrane of low molecular weight (MWCO of 100 g/mol) is necessary preventing the oligosaccharide degradation products like glucose, isomaltose, … [13], to leave the sample chamber. In Figure 6, the evolution of the swelling pressure of a degrading dex-MA hydrogel (DS of 4.0; 20% (w/w)) is plotted as a function of degradation time. The dextranase concentration is 0.25 U/g gel and 0.5 U/g gel, respectively. Over the whole degradation period no degradation products were analytically detected (for more details see [12]) in the sample chamber. This indicates that no leakage of the low molecular weight degradation products occurred. In Figure 6, all curves show a saturating non-linear exponential increase in swelling pressure as a function of degradation time. This is conform our expectations as during the hydrogel degradation the increase in number of reducing oligosaccharides shows an identical profile (data not shown). Increasing the dextranase concentration results in a faster (two times) and a higher swelling pressure profile.

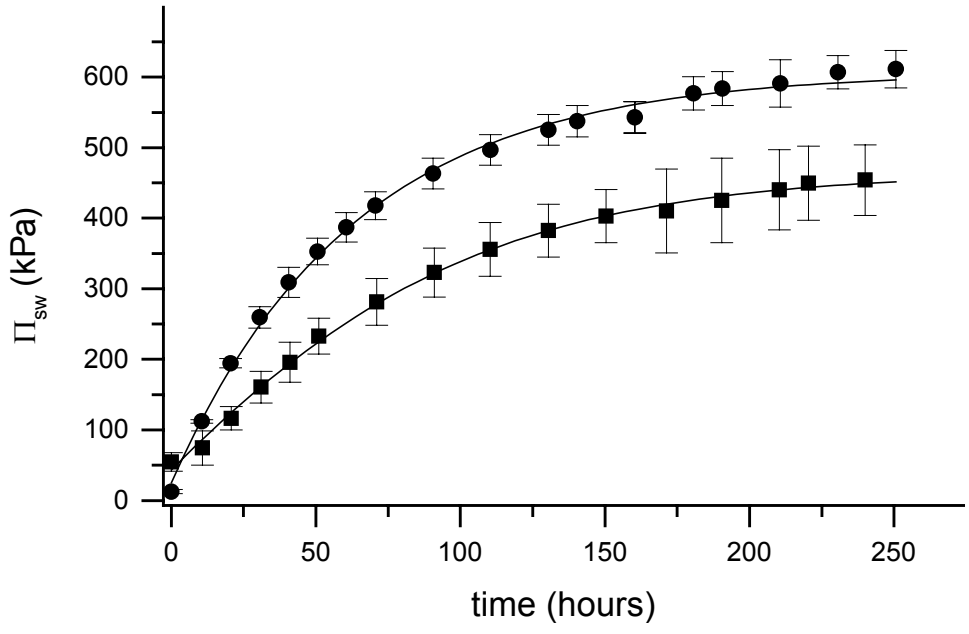

Figure 6. Pressure measurement of enzymatic degrading dex-MA hydrogels (DS4.0; 20%(w/w)) in the osmometer. Influence of enzyme concentration is represented ((■) 0.25 U/g gel and (●) 0.50 U/g gel). Measurements are performed at 37°C. The data are an average of at least two repeats.

Besides the degradation of dex-MA/dextranase hydrogels also the degradation of the dex-HEMA hydrogels was evaluated inside the osmometer. Unfortunately, dex-HEMA hydrogel degradation was inhibited and could not be followed inside the device. Compared with unconfined hydrogels, less water is present when the gel is confined inside the sample chamber. This might explain why the dex-HEMA hydrogel degradation did not occurred in the time followed (about 100 days).

Comparison between the osmometer and osmotic deswelling

In brief, the pro and contra's of both methods (i.e. the swelling pressure device and the osmotic deswelling) are discussed. Osmotic deswelling is an 'indirect' method for measurement of the swelling pressure of hydrogels. The hydrogels are first allowed to swell during degradation and are only afterwards deswollen towards the initial concentration (volume) of the non-degraded gel. Hence, the osmotic deswelling method provides pressure versus concentration plots making prediction of the swelling pressure of the membrane surrounded hydrogels possible, even when stretching of the membrane occurs. However, the osmotic deswelling method is extremely time and product consuming. In addition, at the gel-sol transition no swelling pressure measurements can be performed resulting in a loss of information at the end of the hydrogel degradation. However, compared with the osmometer, no expensive equipment is necessary. In the osmometer, the swelling pressure of the hydrogel is measured in a 'direct' way. During the measurement the hydrogel is confined to a constant volume and mimics the coated hydrogels as we assumed no stretching of the membrane. This might be not the case. The moulding of the hydrogel in the device is critical, especially inclusion of bubbles should be prevented. Hydrogels which tend to shrink after moulding (e.g. dex-HEMA DS7.5; 20% (see Chapter 3)) cannot be measured in the device.

CONCLUSIONS

A new type of device is proposed that uses the sensitivity and reliability of the pressure transducer to measure the swelling pressure of complex systems as biodegradable hydrogels. The dimensions and material characteristics of the osmometer were optimized for the measurement of degrading hydrogels. In the proposed device, degradation induced changes in the osmotic swelling pressure of pharmaceutical hydrogels are measured by confining the hydrogel in an enclosure between a rigid semi-permeable membrane and the diaphragm of a pressure transducer. Sensor response time ranges between 3 and 6 hours for polymer solutions and polymer hydrogels, respectively. The device is validated by measurement the pressure of dextran and PEG solutions with known osmotic pressure. The pressure values are in good agreement with literature data. In order to further validate

the device the swelling pressure of a dex-MA (DS3.1; 25%) hydrogels is measured and compared with osmotic deswelling data of the same gel. It takes about 64 ± 10 kPa of mechanical pressure and 69 kPa of osmotic stress to keep the hydrogel volume fixed. Therefore both methods are considered to be in acceptable agreement. The device is able to monitor constant changes in osmotic properties of the enzymatic degrading hydrogel, i.e. dex-MA/dextranase gel. However, degradation of the dex-HEMA gels was inhibited inside the device and changes in osmotic properties could not be followed. In conclusion, depending on the type of hydrogel system and depending on its degradation mechanism the osmometer is a suitable alternative for the osmotic deswelling technique.

ACKNOWLEDGEMENTS

Mies van Steenbergen is gratefully acknowledged for the synthesis of dex-(HE)MA. Mr. Velghe from Atlantic Engineering (Nazareth, Belgium) is acknowledged for the support and development of the osmometer. Brecht Stubbe is acknowledged for the support in the data-logging part. The author also thanks Roos Vandenbroecke for the drawing of the device.

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5

Influence of the degradation mechanism on the swelling pressure of degrading hydrogels

Abstract

This study compares the behavior of dextran-based hydrogels that degrade through different mechanisms. The major aim is to investigate the influence of the degradation mechanism on the swelling pressure of the degrading hydrogels. The release of degradation products, the mechanical and swelling properties and swelling pressure of the degrading gels are measured. Two types of dextran-based hydrogels are investigated. Dextran methacrylate (dex-MA) hydrogels that are degraded by entrapped dextranase serve as a model for hydrogels that degrade by hydrolysis of the polymer backbone. Dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels are used as a model for hydrogels that degrade at their cross-links. The release of degradation products but especially the swelling pressure profile seems to be strongly dependent on the mechanism underlying the degradation of the gels. In case the dextran gels are degraded at their backbone the swelling pressure increases rather continuously, in case they are degraded at the cross-links it increases more discontinuously as a sudden increase occurs when the gels are (nearly) completely degraded. This study reveals that the increase in swelling pressure in degrading dex-MA/dextranse gels is (nearly) completely attributed to an increase in osmotic pressure. However, in degrading dex-HEMA gels the increasing swelling pressure seems to be attributed to the decrease in elastic pressure (i.e. elasticity) of the gels. Indeed, during a substantial period the osmotic pressure of degrading dex-HEMA gels does not change. At complete degradation the maximal swelling pressure is obtained and equals the osmotic pressure of the solution of degradation products. A much higher maximal swelling pressure is obtained when the gels are degraded at their backbone than when degraded at their cross-links.

INTRODUCTION

There is a general interest in biodegradable polymer networks for use in controlled drug delivery devices.[1] In addition, there is also a major interest in pulsed drug delivery devices which release the drug at pre-programmed times. Several concepts show potential to release drugs in a pulsed way.[2] However, many of the proposed systems have several draw-backs like initial drug release (the so called 'burst release'), sustained like release instead of pulsed release, … Our research group aims to develop '*exploding microcapsules*' for pulsed drug delivery.[3;4] We envision a delivery device that consist of a micron sized degradable gel particle surrounded by a water permeable membrane. As the microgel degrades the internal pressure rises resulting in a burst of the membrane followed by a fast drug release. The microgels in our study are degradable dextran-based hydrogels. As membrane components we investigate lipids and polyelectrolytes. The exploding microcapsules which we try to design should show a behavior comparable with the osmotic bursting of red blood cells when immersed in a hypotonic solution. The difference being that the bursting in the microcapsules relies on the increase in swelling pressure of the degrading hydrogel core which, on its turn, depends on the degradation kinetics of that microgel.

With this purpose in mind it is extremely important to be able to evaluate the degradation of the (dextran) microgel core and, especially, to understand which parameters govern its swelling pressure increase. In literature, mechanical properties, degree of swelling and release of degradation products are typically reported in studies on degradable hydrogels.[5-9] In a previous paper of our group we also measured the *swelling pressure* of degrading hydrogels. We developed therefore a swelling pressure meter similar to the device described by Han et al.[10] Furthermore we modified the osmotic deswelling technique as reported by Horkay et al.[11] to measure the swelling pressure of the dextran-based hydrogels during degradation.

Degradation of polymer networks can occur by different mechanisms: (i) by hydrolysis of side chains or pendant groups (ii) by cleavage of the polymeric backbone and (iii) by cleavage of labile groups in the cross-links. As shown in Figure 1, two types of dextran-based hydrogels, which degrade by different mechanisms, are investigated in this study: dextran methacrylate (dex-MA) hydrogels that degrade by cleaving the dextran backbone by the endoenzyme dextranase (entrapped within the hydrogel during polymerisation) and dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels that degrade by hydrolysis of the cross-links that contain hydrolysable carbonate esters (between the methacrylate group and the dextran).[12;13]

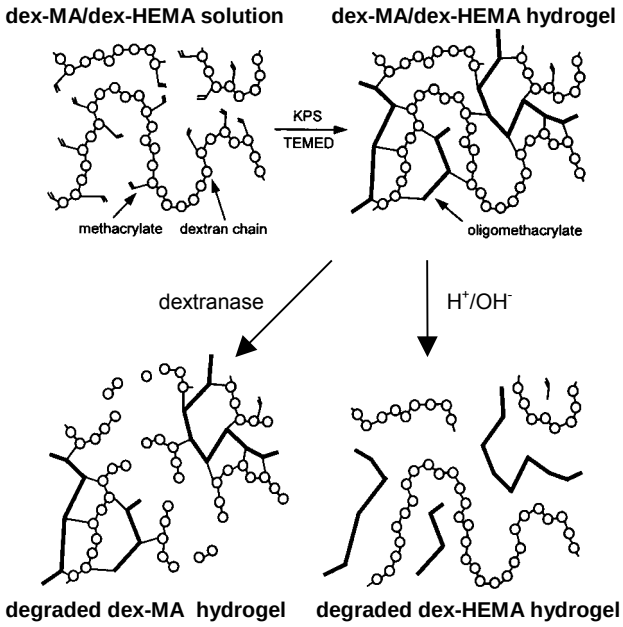


Figure 1. Schematic representation of the polymer network in dex-MA and dex-HEMA hydrogels. (o) represents glucose units in the dextran chains, (⌐) represent the reactive methacrylate or hydroxyethylmethacrylate groups in dex-MA and dex-HEMA, respectively. The enzymatic hydrolysis by dextranase of the dextran chains in dex-MA gels and the hydrolysis of the cross-links in dex-HEMA gels is also illustrated.

The aim of this study was threefold. First of all, we aimed to evaluate how the degradation mechanism of the hydrogels influence the release of the degradation products, the mechanical and swelling properties of the gels. Secondly, we aimed to reveal how the swelling pressure of the gels changes during degradation and how this depend on the mechanism behind the degradation. Thirdly, based upon the release of the degradation products and the elasticity of the degrading gels we aimed to calculate the swelling pressure of the degrading gels. The calculated swelling pressure values were then compared with the experimentally measured values.

EXPERIMENTAL SECTION

Preparation of the hydrogels

Dex-MA and dex-HEMA were synthesized and characterized as described in detail elsewhere.[14-16] Dex-MA and dex-HEMA were synthesized from dextran (from *Leuconostoc ssp*, Merck) with an average molecular weight of 19 000 g/mol. The degree of substitution (DS) of the dex-MA and dex-HEMA, which is the number of (HE)MA groups per 100 glucose units, equalled 3.1 and 2.9 for dex-MA and dex-HEMA gels, respectively.

Dex-MA and dex-HEMA hydrogels were prepared by radical polymerization of aqueous dex-MA and dex-HEMA solutions, respectively. The solutions were prepared by dissolving the polymer in phosphate buffer (PB: 10 mM Na_2HPO_4, 0.02% sodium-azide, adjusted with 1 N hydrochloric acid to pH of 7.0). The polymerization reagents were N,N,N',N'-tetramethylene-ethylenediamine (TEMED; 20% v/v in deoxygenated PB, pH adjusted to 8.5 with HCl) and potassium persulphate (KPS; 50 mg/mL in deoxygenated PB). The gelation of dex-MA and dex-HEMA solutions was initiated by adding 50 µL TEMED solution and 90 µL KPS solution (per gram hydrogel). Gelation occured at 4°C.

When "dex-MA/dextranase" gels were prepared, prior to addition of the gelation reagents, a dextranase solution (D-1508 Sigma; diluted to 10 U/ml in 10 mM PB pH of 7.0; one unit will delivers 1 µmol of isomaltose per min at pH of 6.0 at 37°C) was added to the dex-MA solution (cooled to 4°C). Throughout this work the dextranase concentration in the dex-MA gels was always 0.2 U/g gel.

"Dex-HEMA/dextran" hydrogels were made by radical polymerization (as described above) of solutions containing both dex-HEMA and dextran (19 000 g/mol, Merck).

Rheological characterization of the hydrogels

Rheological measurements on the hydrogels were performed by an AR1000-N controled stress rheometer from TA-Instruments according to a method described in detail by Meyvis et al.[6]

Characterization of the degradation products of the hydrogels

Individual hydrogel slabs were submerged in 10 mL phosphate buffer (pH of 7.2) and stored at 37°C. Samples (2.5 ml) were taken at regular time intervals and replaced by fresh buffer. Twice a day the containers were slightly shaken.

The concentration of reducing oligosaccharides released from the degrading dex-MA hydrogels was determined spectrophotometrically with Sumner reagent as described by Franssen et al.[17] In brief, 1.5 mL of Sumner reagent (0.2 g/mL sodium potassium tartrate, 10 mg/mL dinitrosalicylic acid, 10 mg/mL sodium hydroxide and 2 mg/ml phenol) and 100 µL freshly prepared sodium sulfite solution (30 mg/mL) were added to 1 mL sample. This mixture was incubated for 15 minutes at 95°C. After cooling to room temperature, the absorbance was measured at 620 nm (Biochrom 4060 spectrophotometer). The concentration of reducing oligosaccharides was calculated from a calibration curve using glucose solutions as a reference.

The concentration of dextran chains released from the degrading dex-HEMA gels was measured by differential refractive measurements as described by Stubbe et al.[3]

Swelling experiments

To characterize the swelling behaviour of the degrading dextran hydrogels, they were weighed immediately after preparation (w_0) and at several time points during their degradation (w_t). The swelling ratio (Q) was calculated as follows:

$$Q\,(\%) \;=\; \frac{w_t - w_0}{w_0} \times 100 \qquad (1)$$

Swelling pressure measurements

The swelling pressure (Π_{sw}) of the dex-MA/dextranase gels was determined by a home-built 'swelling pressure meter'. This device consists of a callibrated transducer (Honeywell, allows to measures Π_{sw} up to 7 atmospheres), a sample chamber (which contains the gel, volume 4 mL) and a buffer chamber (volume 15 mL). The chambers are separated by a semi-permeable membrane (Spectra Por®, M_w cut-off 100 g/mol) supported by a porous Bekipor® frame which is further supported by a teflon perforated cylinder. The membrane is permeable to water but impermeable to oligosaccharides being the degradation products of dex-MA/dextranase gels. To measure Π_{sw} of degrading dex-MA/dextranase gels the sample chamber (cooled at 4°C) was filled with the dex-MA/dextranase solution during gelation. The buffer chamber (also cooled at 4°C) was filled with PB at pH of 7.0 containing equivalent concentration of KPS and TEMED as present in the dex-MA/dextranase gels. After moulding, dex-MA/dextranase hydrogels were allowed to equilibrate at 4°C (no substantial degradation of the gels occured) during 12 hours. To start degradation the temperature of the device was increased to 37°C and Π_{sw} values were registered as a function of time.

The swelling pressure meter seemed to be less suited to measure Π_{sw} of degrading dex-HEMA gels. We observed that a dex-HEMA gel in the swelling pressure meter degraded slower than the same gel submerged in buffer. The reason is still unclear. Therefore, to measure Π_{sw} of the dex-HEMA gels as a function of degradation time we worked as follows. The dex-HEMA gels were allowed to degrade during different times in phosphate buffer (pH of 7.0). Then the swelling pressure of the (partially) degraded dex-HEMA gels was measured by 'osmotic deswelling' as described in detail by Horkay and Zrinyi.[18] Deswelling was achieved by enclosing the (partially) degraded dex-HEMA gels in dialysis bags (Medicell, M_w cut-off 12-14 000 g/mol) and submerging them into poly(ethylene)glycol (PEG, Merck, M_n of 20 000 g/mol) solutions of known osmotic pressure. Equilibrium swelling was attained within 7 days. At equilibrium, the swelling pressure of the (partially) degraded dex-HEMA gel (in the dialysis bag) equaled the osmotic pressure exerted by the PEG solution outside. As the deswelling in the PEG-solutions occurred at 4°C, the dex-HEMA gels did not further degrade during the osmotic deswelling step (7days).

RESULTS & DISCUSSION

Dex-MA/dextranase hydrogels degrade through hydrolysis of the polymer backbone. As shown in Figure 1, the endodextranase, entrapped within the dex-MA network during polymerization, hydrolyzes the dextran chains of the polymer network. After a first cleavage of a dextran chain, the remaining strands (dangling ends) can be further degraded if they are long enough to fit within the active site of the dextranase.[19] Franssen et al. identified (by electrospray mass spectroscopy) the products obtained by enzymatic degradation of the dex-MA gels. E.g. for a dex-MA/dextranase hydrogel of DS 4.0, they showed a variety of degradation products which are mainly oligosaccharides like glucose, isomaltose, … and monomethacrylated iso-maltotriose.[19] In opposite, degradation of dex-HEMA gels through hydrolysis of the cross-links results in more well defined degradation products. As shown in Figure 1, hydrolysis of the carbonate ester cross-links results in the release of dextran chains and low molecular weight oligohydroxyethylmethacrylate fragments (from the cross-links).

Figure 2 compares the release of degradation products, the rheological properties and the swelling behavior of degrading dex-MA/dextranase and degrading dex-HEMA gels, respectively. The dex-MA concentration in the (non-swollen) dex-MA/dextranase hydrogel was 25% (w/w) while the DS of dex-MA was 3.1. Similarly, the dex-HEMA concentration in the (non-swollen) dex-HEMA hydrogel was 25% (w/w) while the DS of the dex-HEMA was 2.9.

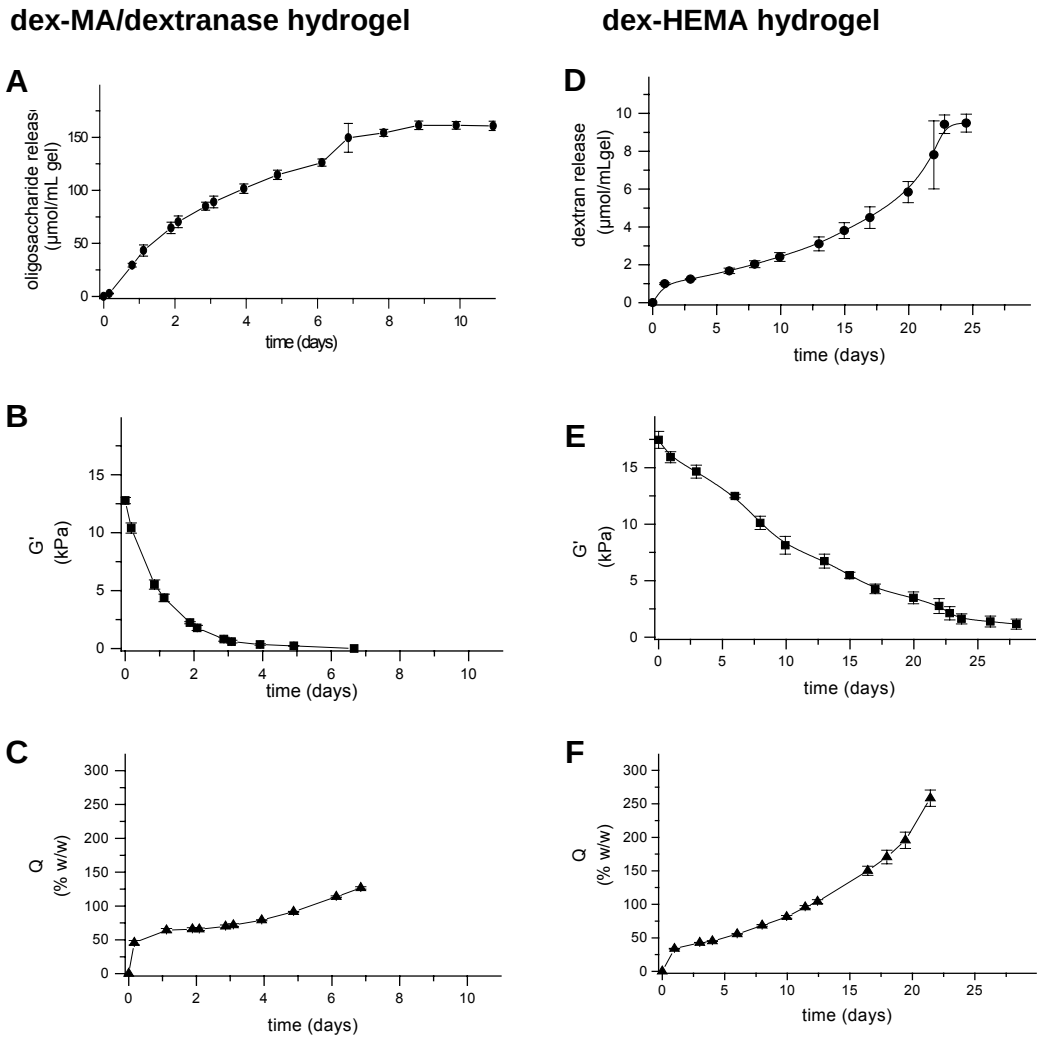


Figure 2. Cumulative release of the degradation products (i.e. oligosaccharides) **(A)**, the elastic modulus (G') **(B)** and the swelling (Q) **(C)** of degrading dex-MA/dextranase hydrogels. The dex-MA concentration was 25% (w/w), the DS was 3.1 and the dextranase concentration was 0.2U/g gel. Cumulative release of the degradation products (i.e. dextran) **(D)**, the elastic modulus (G') **(E)** and the swelling (Q) **(F)** of degrading dex-HEMA hydrogels. The dex-HEMA concentration was 25% (w/w) and the DS was 2.9. All data are the average of three independent measurements.

Figure 2A shows the amount of reducing oligosaccharides released from the degrading dex-MA/dextranase gel. A gradual increase in the amount of degradation products is observed as degradation proceeds: initially large amounts are released per time unit while the release rate slows down as degradation proceeds. This can possibly be explained as follows: the longer the degradation, the more likely that dextranase was released from the (degrading) dex-MA gel either in free form or bound to degradation products. Figure 2B shows the elasticity of the degrading dex-MA/dextranase gel. A major exponential decrease in G' occurs during the initial part of the degradation: already after 2 days the hydrogel lost the majority of its elasticity while after 7 days the gel became a solution. G' only decreases when the dextranase degrades elastic network chains in the dex-MA gels. In opposite, the release rate of reducing oligosaccharides, is influenced by the action of the dextranase on both elastic and non-elastic dextran chains present in the hydrogel. The swelling of the degrading dex-MA/dextranase hydrogel is shown in Figure 2C. The first point (at t = 0) concerns the hydrogel just after cross-linking, before any degradation occured (swelling ratio equals zero). The high swelling ratio after 1 day is mainly due to the change of the gel from its relaxed state into its swollen state (upon submerging in phosphate buffer). It was striking to observe that during the first days of degradation, the swelling ratio of the dex-MA/dextranase hydrogels hardly changed while the G' of the hydrogels decreased considerably. This is explained as follows and schematically represented in Figure 3A. When an elastic network chain of the dex-MA gel is degraded by dextranase, the concentration of *elastic network chains* decreases and thereby also G'. However, due to the high functionality of the cross-links, the concentration of *elastic cross-links* will remain the same during a substantial time of the degradation process. As a consequence, the average molecular weight between the remaining cross-links is also unaltered, which does not allow the network to expand. [7]

Figure 2D shows the release of degradation products (i.e. dextran chains) from the dex-HEMA gels. In stead of an immediate release as observed for dex-MA/dextranase gels (Figure 2A), a rather retarded release of degradation products was observed for the dex-HEMA gels (Figure 2D): after the release of the sol fraction (being dex-HEMA chains not attached to the network during cross-linking), a lag phase occurs while towards the end of the degradation the release clearly occurs faster. The lag phase is explained by the fact that all cross-links connecting a single dex-HEMA chain to the network have to be broken before the chain can be released. The amount of dextran released at the end of the degradation equaled the initial amount of dex-HEMA used at cross-linking. A similar release of degradation products was observed by Lee et al. in their study on poly(aldehyde guluronate) hydrogels which also degrade through degradation of the cross-links.[20] Compared with the dex-MA/dextranase gels, G' decreases more continuously during degradation of dex-HEMA hydrogels (Figure 2E). As illustrated in Figure 3B, the hydrolysis of each cross-link in a dex-HEMA gel decreases both the elastic chain and cross-link concentration which is reflected in the G'. However, we should be careful when

comparing G' of degrading dex-MA/dextranase and dex-HEMA gels, as the degradation rate of the dex-MA/dextranase gels is strongly dependent upon the enzyme concentration. In Figure 2F the swelling behavior of the degrading dex-HEMA gels was followed. After an initial swelling observed upon submerging the relaxed dex-HEMA gel in buffer, the dex-HEMA gels swelled continuously during degradation. Note that a lag time in swelling was observed for the dex-MA/dextranase gel (Figure 2C). As illustrated in Figure 3B, the hydrolysis of each cross-link on a dex-HEMA chain results in a longer network chain between the remaining cross-links. Consequently, these longer chains can expand which explains the continuous swelling of the hydrogels and which also explains the higher swelling of the dex-HEMA gels compared to the dex-MA/dextranase gels (Figure 3B).[7]

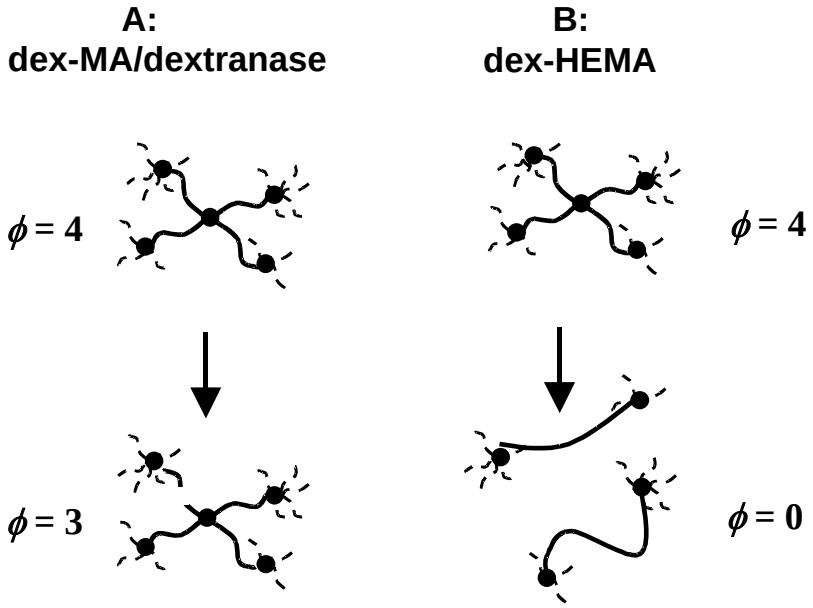


Figure 3. Schematic representation of the degradation of respectively a dex-HEMA network by dextranase (A) and a dex-HEMA network which degrades through hydrolysis of the cross-links (B). (ϕ) refers to the functionality of the cross-links (•).

Besides being interested in how the degradation mechanism influences the release of degradation products, the rheological properties and the swelling behavior of the gels, we were especially interested to know how the swelling pressure of the two types of degrading gels builds up during degradation. Figure 4 shows that the swelling pressure of dex-MA/dextranase gels increases gradually during degradation. Besides Π_{sw}, Figure 4 also shows the osmotic pressure (Π_{osm}) and the elastic pressure (Π_{el} = G') of the degrading dex-MA/dextranase hydrogels. Π_{osm} was calculated from the experimentally measured Π_{sw} and Π_{el} using the following equation:

$$\Pi_{sw} = \Pi_{osm} - \Pi_{el} \quad (1)$$

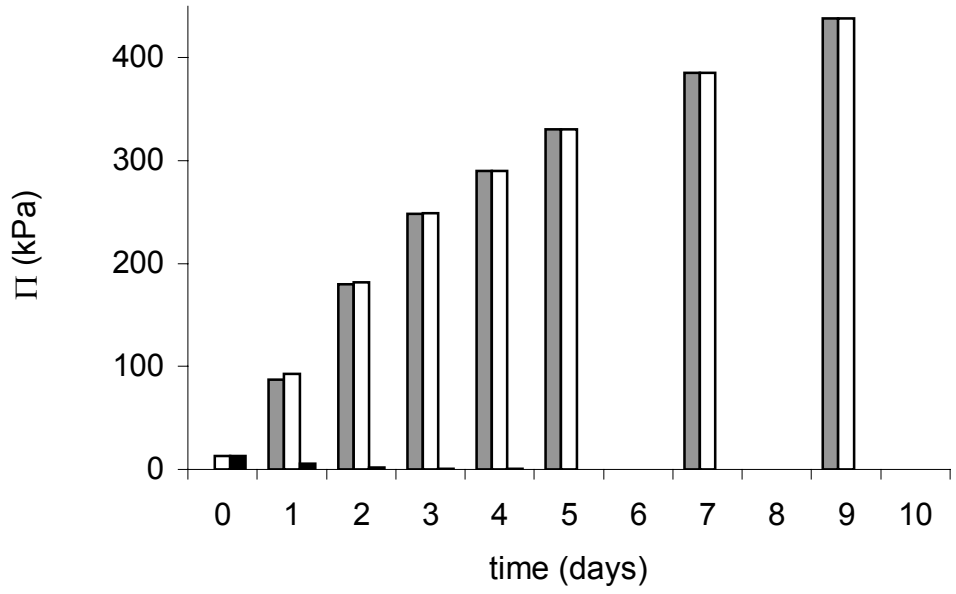
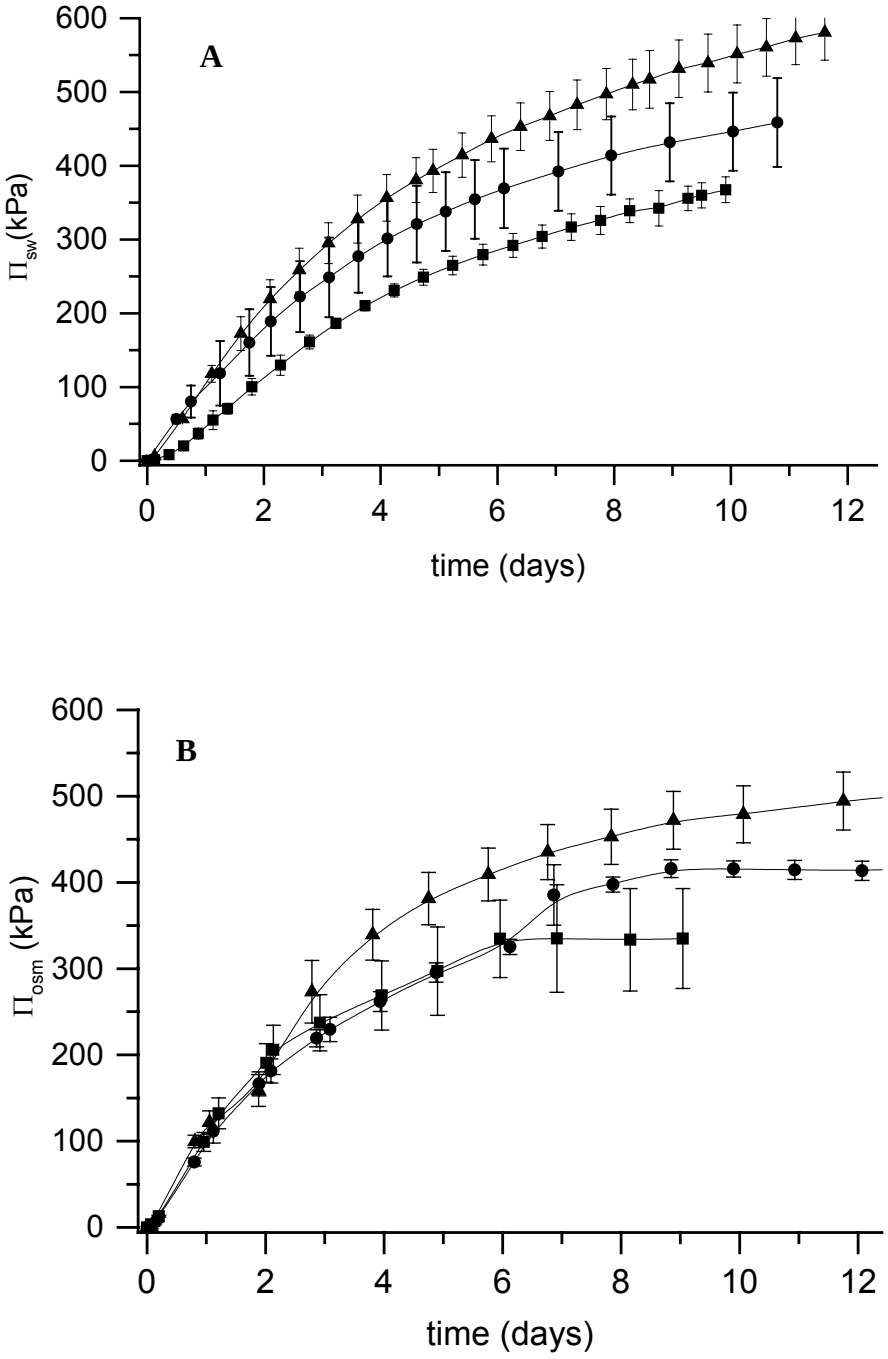


Figure 4. Osmotic pressure (open bars), elastic pressure (black bars) and swelling pressure (grey bars) of degrading dex-MA/dextranase hydrogels (DS3.1;25% and 0.2U/g gel dextranase).

Clearly, the increase in Π_{sw} mainly originates from the increase in osmotic pressure and only slightly from the decrease in elastic pressure: the contribution of the elastic pressure to the swelling pressure of degrading dex-MA/dextranase gels seems to be neglectable. In other words, the change in osmotic pressure of the dex-MA/dextranase gel nearly equals the change in swelling pressure. One can wonder why the *swelling profile* in Figure 2C does not follow the *swelling pressure profile* of the dex-MA/dextranase gels in Figure 4. Clearly, in the swelling measurements in Figure 2C the degradation products were allowed to diffuse out of the gels and, consequently, did not contribute to the swelling profile. However, in the swelling pressure measurements in Figure 4 the degradation products stayed in the gel (as they could not diffuse through the semi-permeable membrane in the swelling pressure device) and contributed to Π_{sw}.



Figuur 5A. Swelling pressure (as measured by the swelling pressure device) of degrading dex-MA/dextranase hydrogels (DS 3.1; 0.2U/g gel dextranase) with different dex-MA concentrations: 20% (■), 25% (●) and 30% (▲). **B.** Osmotic pressure of degrading dex-MA/dextranase gels as calculated from the release of oligosaccharides (see Figure 2A) using equation 2. The data are the average of three independent measurements**.**

We further focused on the swelling pressure of degrading dex-MA/dextranase gels by varying the dex-MA concentration of the gels. Figure 5A shows the experimental results obtained with the swelling pressure device. The maximal swelling pressure, which is obtained when the dex-MA/dextranase gels are totally degraded, was higher for more concentrated gels. This was expected as, the higher the initial dex-MA concentration at cross-linking the higher the concentration of degradation products which increases Π_{osm} and thus Π_{sw}. Figure 5B shows the osmotic pressure of the degrading dex-MA/dextranase hydrogels in Figure 5A as calculated using the Van 't Hoff law:

$$\Pi_{osm} = c.R.T \qquad (2)$$

where c is the concentration (in mol/L) of oligosaccharides in the dex-MA/dextranase gels as calculated from the oligosaccharide release experiments in Figure 2A. R is the gas constant and T is the absolute temperature. Comparing Figure 5A and 5B reveals that the Π_{osm}-profile, as calculated with equation 2, fairly corresponds to the experimentally obtained Π_{sw}-profile in Figure 5A. This confirms that the swelling pressure of degrading dex-MA/dextranase gels can be easily predicted from their osmotic pressure as calculated from the amount of oligosaccharides released during degradation.

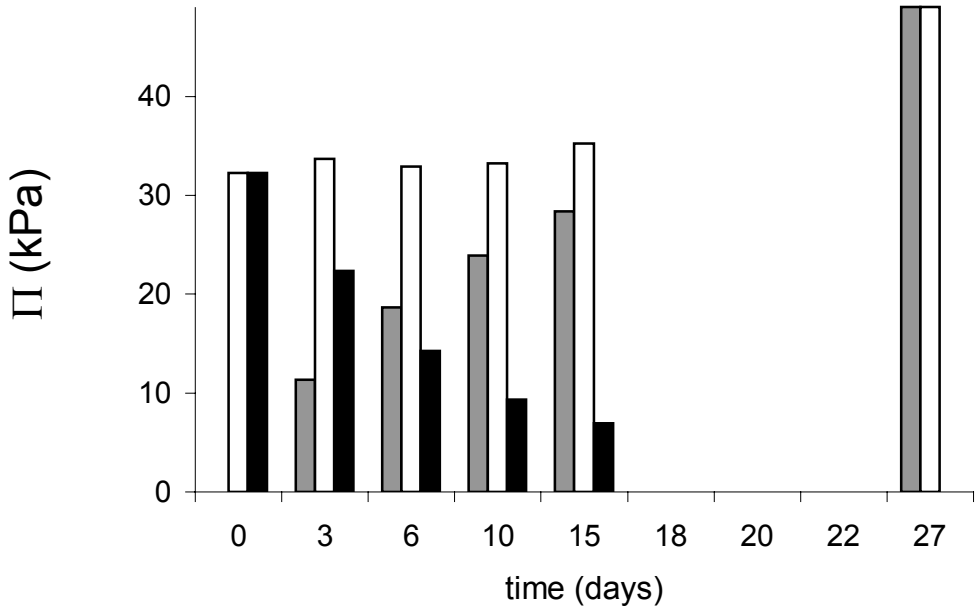


Figure 6. Osmotic pressure (open bars), elastic pressure (black bars) and swelling pressure (grey bars) of degrading dex-HEMA hydrogels (DS2.9;25%). After 15 days the dex-HEMA gels became to weak to manipulate and to make swelling pressure measurements. After 27 days, when the gel turned into a complete polymer solution, a final measurement could be performed.

Figure 6 shows that the Π_{sw}-profile of dex-HEMA gels totally differs from the one of dex-MA/dextranase gels. In the first 15 days of the degradation process a gradual increase in swelling pressure was observed. However, the increase in Π_{sw} is completely attributed due to the decrease in elastic pressure as no significant change in Π_{osm} can be observed. Hence, the elastic contribution to Π_{sw} cannot be neglected as is the case for dex-MA/dextranase gels. Secondly, to the end of the degradation (when the gel becomes a solution), the swelling pressure suddenly increases which seems to originate from a sudden increase in osmotic pressure at the 'gel-sol' transition. Similar observations have been reported for other polymer/solvent systems.[21;22]

In order to explain the osmotic pressure properties of degrading dex-HEMA gels, dex-HEMA gels were made in the presence of free dextran chains. As during degradation of the dex-HEMA gels dextran is released, the dex-HEMA/dextran gels mimic 'partially degraded dex-HEMA gels'.[4] In Figure 7 the osmotic pressure of a non-degraded dex-HEMA gel (25% dex-HEMA DS 2.9) and of a dex-HEMA gel containing free dextran chains (12.5% dex-HEMA DS 2.9 and 12.5% free dextran) is shown. Despite the fact that a high amount of free dextran chains is present in the dex-HEMA/dextran gel, there is no significant difference in osmotic pressure of these gels. Hence, the dextran chains present in the dex-HEMA gel do not behave as free chains but as additional network chains. Based upon light scattering experiments, Kloster et al.[23-25] reported that the presence of a gel matrix reduces the entropy and hence the osmotic pressure of the free chains present in that gel matrix. This probably explains the constant value of Π_{osm} in degrading dex-HEMA gels (Figure 5): during degradation, the amount of free dextran chains in the dex-HEMA gels increases, however, they do not increase the osmotic pressure as they behave as additional network chains in the remaining dex-HEMA network.

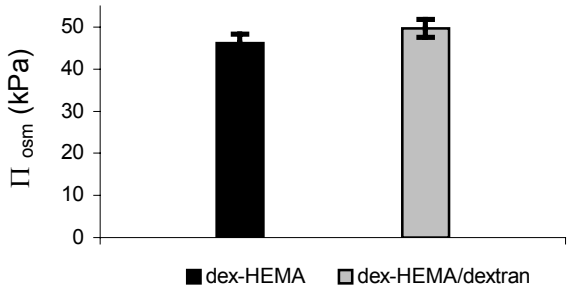


Figure 7. Osmotic pressure of a non-degraded dex-HEMA gel (DS2.9; 25%) and a similar dex-HEMA/dextran gel containing 12.5% dex-HEMA (DS2.9) and 12.5% dextran.

Besides the differences in shape of the swelling pressure curves (Figures 4 and 6), the maximal value Π_{sw} (which is obtained at complete degradation) significantly differs between dex-HEMA and dex-MA/dextranase gels. This is explained as follows. The molecular weight of the dextran chains(being the degradation products in the dex-HEMA gels) is much higher than the molecular weight of the oligosaccharides (being the degradation products of dex-MA/dextranase gels). Consequently, for dex-HEMA gels and dex-MA gels with the same initial (respectively dex-HEMA and dex-MA) concentration at cross-linking, the molar concentration of the degradation products is much higher in the solution obtained by degrading dex-MA gels than in the solution obtained from dex-HEMA gels.

As explained above, for dex-MA/dextranase gels the swelling profile in Figure 2C does not follow the swelling pressure in Figure 4. Although, for dex-HEMA gels the *swelling profile* (Figure 2F) does resemble the *swelling pressure profile* (Figure 6). One could wonder, however, why the sudden increase in *swelling pressure* is not reflected in a sudden volume expansion of the gel. This is explained by the fact that the increase in *swelling pressure* occurs at the very end of the degradation process i.e. when the gel becomes a solution. At this time swelling measurements are no longer feasible.

CONCLUSIONS

This study compares the behavior of dextran-based hydrogels that degrade through different mechanisms. On the one hand dex-MA hydrogels that are degraded by entrapped dextranase were used, being a model for hydrogels that degrade by hydrolysis of the polymer backbone. On the other hand, dex-HEMA hydrogels were studied, as a model for hydrogels that degrade by hydroysis of the cross-links. Upon degradation of dex-MA/dextranase gels the degradation products, being oligosaccahrides, were gradually released from the gels, an exponential decrease in G' and a minor increase in swelling was observed. In degrading dex-HEMA gels the release of degradation products, being dextran chains, only began after an initial lag phase, followed by a rather fast release of degradation products. A higher swelling was observed for degrading dex-HEMA gels compared to dex-MA/dextranase with the same dextran concentration.

The main focus of this study was to investigate the swelling pressure profile of the degrading dextran gels and to show how the degradation mechanism influences the change in swelling pressure. Typically, for dex-MA/dextranase gels a gradual increase in swelling pressure profile was observed. For dex-HEMA gels a sudden increase in swelling pressure at the end of the degradation occured, an interesting feature to use dex-HEMA gels as core in the exploding microcapsules outlined in the introduction. From the release of the degradation products and the elasticity of the degrading dex-MA/dextranase gels the swelling pressure was also calculated and seemed to be in good agreement with the

experimentally measured values. This allowed to conclude that the swelling pressure of degrading dex-MA/dextranase gels can be well predicted from their osmotic pressure, as calculated from the release studies. In other words, the increase in swelling pressure in degrading dex-MA/dextranse gels is (nearly) completely governed by the increase in osmotic pressure. However, in the initial period of the degradation the increase in swelling pressure of the dex-HEMA gels seemed to be completely attributed to the decrease in elasticity as we observed minor changes in osmotic pressure in that period.

Finally, the maximal swelling pressure which can be obtained by degrading the gels equals the osmotic pressure of the corresponding solution of degradation products. Consequently, as the degradation products are oligosaccharides in dex-MA/dextranase gels while they are dextran chains in dex-HEMA gels, this maximal value of the swelling pressure is higher for dex-MA/dextranase gels than for dex-HEMA gels with the same dextran concentration at cross-linking.

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6

Self-exploding microparticles

Abstract

Self-exploding microparticles show potential for advanced delivery of certain therapeutics. This study evaluates (**i**) whether degrading dextran hydroxyethyl methacrylate (dex-HEMA) microgels can become coated by a lipid membrane and (**ii**) whether the surrounding membrane can be suddenly ruptured by the increasing swelling pressure of the degrading microgel. We found that adsorption of charged liposomes to oppositely charged microgels allows efficient coating of the microgels; microparticles with a 'core-shell' structure were clearly obtained. Especially, we could experimentally confirm that the swelling pressure increase in degrading dex-HEMA microgels can suddenly destroy its surrounding lipid membrane. As no external trigger is needed to rupture the membrane 'self-exploding' microparticles were obtained in this way.

INTRODUCTION

Pharmaceutical research strives to design systems that deliver drugs according to therapeutic needs. Conventional drug delivery systems release the drug in a rather fast way. This results in a sharp increase in drug concentration in the blood. After peaking the drug concentration often decreases so fast that the time spent in the therapeutic concentration range is relatively short. To overcome this and to avoid multiple dosing, controlled drug delivery scientists designed tablets, pumps, implants, patches, ... which provide a continuous drug release over a prolonged period of time. However, there are many applications in medicine where a non-uniform release profile would be more beneficial.[1] For bioactive agents such as hormones (e.g. growth hormones) many have suggested that pulsed release may offer advantages over continuous release.[2-4] Also, a pulsed release pattern could be advantageous for drugs that develop biological tolerance and for drugs that require dosing at night. Devices that give pulses of drugs at well-defined times after injection could be used to provide 'single-shot' vaccines. Such devices could improve vaccination coverage by reducing the number of administrations required to generate immunity.

Very challenging and promising towards multiple pulsed drug delivery from a single implant are the biodegradable polymeric chips under investigation by Langer and co-workers.[5,6] These are poly(lacticglycolic acid) (PLGA) based chips which consist of multiple reservoirs loaded with the drug to be released. Each reservoir is sealed with a PLGA degradable membrane, the molar mass of the PLGA controlling the degradation rate and thus the time at which the drug is released. It has been shown '*in vitro*' that such devices allow multiple pulsed delivery, even over a period of several months. However, the proposed chips are relatively large (around 1 cm in diameter and 0.5 mm thick) and have to be implanted. Injectable (micron sized) devices which could deliver the drug at a programmed time after injection could make a further step in multiple pulsed drug delivery.

It is well known that secretory granules are very efficient in storage and sudden delivery of molecules like e.g. histamine, serotonin, calcium, ...[7,8] Such nano-/microscopic granules consist of a polyanionic biopolymer matrix surrounded by a lipid membrane that prevents the leakage of the entrapped molecules. Release of the stored molecules requires electrochemically stimulated fusion of the granule's membrane with the cell membrane. Hereby small pores are formed through which ions (sodium, potassium from the extracellular matrix) and water can enter the granule. These ions exchange with the stored molecules, swelling of the matrix occurs which results in a rapid release of the molecules through the pores to the extracellular matrix. The secretory granule concept has inspired many scientists. E.g. Kiser et al. proposed a combination of microgel science and lipid chemistry to mimick secretory granules for drug delivery.[9,10] They designed (negatively charged) methacrylic acid based microgels, coated by a lipid bi-layer, which were loaded

with the anticancer drug doxorubicin. Pulsed doxorubicin release was indeed observed upon punching holes in the lipid membrane by electroporation. This way of triggering may be however difficult to realize '*in vivo*'. The attractive concept of Kiser et al. encouraged us to try to design 'self-exploding microparticles' which could store a drug for a desired period and could rapidly release at a certain time *without* an external stimulus. In such a 'programmed' device the release would be completely governed by the inner mechanism of the device.

For this purpose we envisioned micron sized (bio)degradable dextran based gel particles surrounded by a membrane which is permeable to water but impermeable to both the entrapped drugs and the osmotic agents (i.e. the degradation products of the microgel). As membranes lipids[10-13], polymers[14] and polyelectrolytes[15] can be used. However, in this study the dextran based microgels are coated with lipid vesicles. Lipid vesicles have been widely used as models for biological membranes to study permeation, to study lipid protein interactions, … [16-18] As biomembranes are also supported by a polymer network (i.e. the cytoskeleton), our lipid coated microgels are closely related. Pulsed drug release can be obtained as follows from the 'self-exploding microparticles'. After injection, the microgel degrades and its swelling pressure will increase, once high enough, the internal pressure will suddenly rupture the membrane causing an immediate release of the entrapped drugs. The inner mechanism which governs the swelling pressure increase, and thus the time of explosion of the microcapsules, is the degradation rate of the microgel.

Several attempts have been made to coat hydrogels with lipids. *Kiser et al.* sedimented (by centrifugation) drug loaded microgels to a dried lipid film. A very low coatings efficiency was obtained. However, coated and non-coated particles could be separated.[9,10] *Ng et al.* modified the hydrogel surface by inserting lipid anchors at the microgel surface, which promote the self assembly of lipid membranes.[11,12] *Kazakov et al.* made liposomes which where loaded with monomers using sonification. Afterwards gelation was performed with photo-polymerization.[19] All these techniques have several drawbacks, such a s low yield and stability, inhomogeneous coating or not applicable to hydrogel microspheres with a diameter in the range of 1 to 10 µm. *De Geest at al.* recently proved the use of polyelectrolyte coating of hydrogels by using the layer-by-layer technique.[15] Lipid coating based upon electrostatic interactions of oppositely charged microgels and lipids has been proposed as alternative lipid coating technique.

In this study we aimed to prove that 'self-exploding core-shell microparticles' can be designed in which the membrane (made from lipids) can be disrupted by the increasing swelling pressure of the degrading gel core (based on dextran hydroxyethylmethacrylate (dex-HEMA)). Clearly, the tensile strength and permeability of the membrane (which depends on the thickness of the coating and the chemistry of the building blocks) and the osmotic properties of the gel core (that change during degradation and which depend on the polymer composition and the degree of cross-linking) need to be fine tuned.

EXPERIMENTAL SECTION

Synthesis of dex-HEMA

Dex-HEMA was prepared and characterized according to a method described elsewhere.[20] Dextran with a number average molecular weight (M_n) of 19 000 g/mol was used. The degree of substitution (DS i.e. the number of HEMA groups per 100 glucopyranose residues in dextran) was determined[21] by proton nuclear resonance spectroscopy in D_2O with a Gemini 300 spectrometer (Varian). The DS of the dex-HEMA used in this study was 2,5.

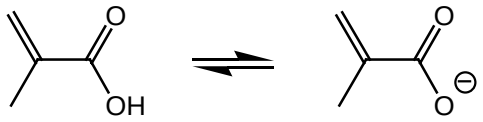
Preparation of the microgels

Dex-HEMA microgels were prepared according to Stenekes et al.[22] In detail, deoxygenated aqueous solutions of dex-HEMA (25% w/w solution) and polyethylene glycol (PEG; 24% w/w solution; Mw 20 000 g/mol) were prepared. The dex-HEMA and PEG solutions were vigorously mixed with a vortex for 1 min under a nitrogen atmosphere to obtain a water-in-water emulsion. A PEG/dex-HEMA ratio of 40 (v/v) was used; the total volume amounted to 5 mL. The resulting emulsion was allowed to stabilize for 10-15 min. Subsequently TEMED (100 µl; pH neutralized with 4 N HCl) and KPS (180 µl of 41 mM) were added to cross-link the dex-HEMA. After gentle mixing the emulsion was incubated without stirring for 30 min at 25°C yielding microgels with an estimated water content of 75% (w/w).[23] Three washing and centrifugation steps with 50 mL Milli-Q water removed the residual KPS and TEMED. The remaining pellet was suspended in 5 mL phosphate buffer (10 mM at pH of 7.0).

To prepare respectively negatively and positively charged dex-HEMA microgels [24], respectively methacrylic acid (MAA; 25µl) or dimethyl aminoethyl methacrylate (DMAEMA; 35µl) was added to the PEG/dex-HEMA mixture described above, just before vortexing it. Figure 1 shows the chemical structure of MAA and DMAEMA, respectively. In this chapter "dex-HEMA-MAA microgels" and 'dex-HEMA-DMAEMA microgels" refer to respectively negatively charged and positively charged microgels.

To prepare fluorescent microgels 4 mg/mL tetramethyl rhodamine B isothiocyanate (TRITC) labeled dextran (M_w of 158 000 g/mol) was added to the dex-HEMA solution used in the preparation of the microgels.

A.



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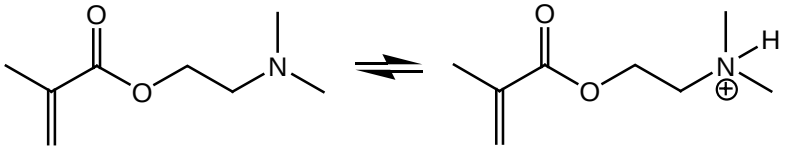

Figure 1. Chemical structure of MAA (A) and DMAEMA (B)

Size distribution of the microgels

The size distribution of the microgels was analyzed by both laser diffraction (Mastersizer, Malvern Instruments) and a transmission light microscope (Eclipse TE300D, Nikon). Home made imaging software was used.

Preparation of the lipid vesicles

Lipid vesicles (liposomes) were prepared as follows. First the lipids were dissolved in chloroform. The chloroform was evaporated at room temperature using nitrogen and the lipid film was further dried under vacuum for 12 hours to remove any remaining chloroform. Large multi-lamellar vesicles were obtained by hydration of the dry lipid film with a carboxyfluorescein (CF) solution (100 mM CF (Fluka), 0.95 M NaCl in 50 mM HEPES at pH of 7.4; 2180 milliosmole (mOsm)) up to a final lipid concentration of 5 mg/mL. Uni-lamellar vesicles were then obtained by extruding the sample eleven times through two stacked polycarbonate filters (100 nm pore size, Nucleopore) using an extruder (Avanti Polar Lipids). The size distribution of the vesicles

was determined by dynamic light scattering (Autosizer 4700, Malvern Instruments). Different lipid compositions were used: **(i)** dioleoyl phosphatidylcholine (DOPC) and cholesterol (CHOL) (molar ratio's 10:0, 9:1, 7:3 and 5:5); **(ii)** stearoyloleyl phosphatidylcholine (SOPC) and dioleoyl trimethylammonium propane (DOTAP) (molar ratio's 9:1, 7:3 and 5:5); **(iii)** SOPC and dioleoyl glycerophosphate (DOPA) (molar ratio 9:1) and **(iv)** SOPC:DOPA:CHOL (molar ratio 4:1:5). All lipids were from Avanti Polar Lipids. After extrusion the unentrapped CF was removed by passing the sample down a Sephadex G-50 column (1.5 × 10 cm) equilibrated with a solution with the same osmotic activity as the CF solution inside the lipid vesicles (i.e. 2180 mOsm).

Osmotically induced lysis of lipid vesicles

Osmotically induced lysis of the lipid vesicles was studied by monitoring the release of the entrapped carboxy fluorescein.[25,26] In the vesicles the fluorescence of the highly concentrated CF is quenched. Only upon release of CF from the vesicles the CF molecules become fluorescent. The vesicles were diluted into buffered saline solutions (i.e. dilutions of 1,1 M NaCl, 50 mM HEPES at pH of 7.4) of different osmotic activities as determined from freezing point depression using an Advanced® Micro-osmometer (Model 3300, Advanced Instruments). Standards of known osmotic activity (50, 290 and 850 mOsm) were also analyzed. The lysis experiments occurred at 25°C. The fluorescence was monitored 1 min after submerging the lipid vesicles in the saline solutions (λ_{ex} 488 nm; λ_{em} 520 nm; Aminco Bowman® Luminescence Spectrometer). Complete CF release was achieved by adding 10 μL Triton X-100 (100 mM) to the sample.

Lipid coating of the dex-HEMA and dex-MA microgels

Lipid vesicles were prepared as described above. The lipid film was hydrated by adding Milli-Q water (final lipid concentration of 1 mg/mL) and sonicated (Bransonic 32, Branson Ultrasonics, 150 Watt) for 5 min. The lipid compositions used to coat the microgels were similar to the ones described above. A small amount (0.05 mol% of the total lipid) of the lipid soluble fluorescent dye cholesteryl BODIPY-FL C12 (λex 504 nm; λem 511 nm; Molecular Probes) was added to make the lipid coating fluorescent.

The (charged) lipid vesicles (500 μL) were mixed with a suspension (200 μL) of (oppositely charged) microgels and incubated for 20 min to allow adsorption of the lipid vesicles to the surface of the microgels. Then the samples were centrifuged (Microfuge 18 Centrifuge, Coulter Beckman) for 5 min at 500 g and the supernatant was removed. The centrifugation and resuspension procedure was repeated three times.

Confocal laser scanning microscopy

Confocal micrographs of the lipid coated microgels were taken with a MRC1024 Bio-Rad confocal laser scanning microscope equipped with a krypton-argon laser. An inverted microscope (Eclipse TE300D, Nikon) was equipped with a water immersion objective lens (Plan Apo 60X, NA 1.2, collar rim correction, Nikon).

Electrophoretic mobility

The electrophoretic mobility of the (lipid coated) microgels was measured by means of a Malvern Zetasizer 2000 (Malvern Instruments). The ζ-potential was calculated from the electrophoretic mobility using the Smoluchowski relation. The dex-HEMA microgel dispersion was centrifuged (1 min. at low speed (500 rpm)) and the measurements were done on the microgels that remained in the supernatant.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) measurements on (lipid coated) microgels were carried out using a Gemini Leo 982 instrument (Digital Scanning Microscope) operating at an accelerating voltage of 3 keV.

RESULTS & DISCUSSION

Preparation and characterization of dex-HEMA microgels

A wide range of techniques is available for the preparation of microparticles. For medical applications it is of paramount importance to produce microparticles under mild conditions. As an example, the use of organic solvents (as in the preparation of the well studied ploy(lactic acid - co - glycolic acid) (PLGA) microspheres) has to be avoided as it may damage e.g. incorporated therapeutic proteins. In this study, the dextran microgels are prepared by a water-in-water emulsion technique based on the immiscibility of the PEG and dex-HEMA solutions.[22,27] As we expected that charged microgels are more suitable towards lipid coating than neutral microgels, we prepared positively (dex-HEMA-DMAEMA) and negatively (dex-HEMA-MAA) charged dex-HEMA microgels. As described in the experimental section, MAA (pKa = 4,5) and DMAEMA (pKa = 8,3) were used to obtain respectively negatively an positively charged dex-HEMA microgels at neutral pH.[24] The incorporation of charged groups into the microgels was verified by

measuring the ζ-potential of the dex-HEMA microgels. Indeed, a negative ζ-potential was observed in case MAA was used, while a positive ζ-potential was measured in case DMAEMA used, indicating the successful charge loading of dex-HEMA microgels (data are further discussed).

The size distribution of the dex-HEMA microgels was characterized by respectively transmission light microscopy and laser diffraction. The results are shown in Figure 2: a number average diameter of 3 μm was obtained by both methods. The rather broad size distribution is due to the nature of the water-in-water emulsion technique.

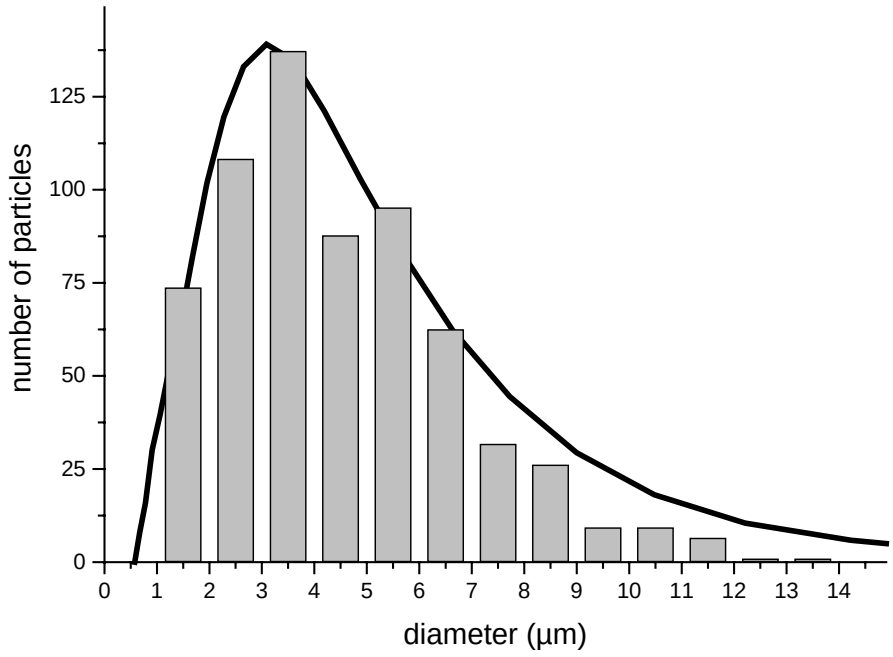

Figure 2. Size distribution of the dex-HEMA microgels determined by laser diffraction (full line) and transmission light microscopy (with n=650).

Lipid coating of dex-HEMA microgels

As outlined in the introduction, to obtain exploding microparticles, a (water permeable) coating which can be ruptured by the swelling pressure of the degrading microgel core is necessary. Technologies which allow to coat the surface of hydrogels are very attractive in biomedicine and pharmacy. A general method for the coating of hydrogel surfaces does, however, not exist so far. Especially, coating hydrogel beads and microparticles requires additional precautions when compared to coating planar supports, a main issue being that the particles need to remain colloidaly stable. Interesting studies on

lipid coating of microgels have been reported in literature.[9-11,24,28] Also the method for lipid coating of microgels reported by Kiser et al., by sedimentation of the microgels on a lipid film by centrifugation, is attractive.[9,10] Although, following this method only a few percentage of the dex-HEMA microgels became lipid coated.

Looking for an elegant and efficient way to coat microgels with a (biocompatible) lipid membrane we came to the idea to make the dex-HEMA microgels positively or negatively charged and to expose them to oppositely charged lipid vesicles (Figure 3). By adding DMAEMA, respectively MAA to the PEG/dex-HEMA emulsion we indeed obtained positively (dex-HEMA-DMAEMA) respectively negatively (dex-HEMA-MAA) charged dex-HEMA microgels, as evidenced from ζ-measurements (Figure 5). The microgels were consequently submerged in a dispersion of oppositely charged lipid vesicles.[29,30] As Figure 4 clearly shows, all the dex-HEMA microgels became lipid coated. Microparticles with a core-shell structure were obtained with lipids only at the surface of the microgels and not in the interior. As Figure 3 illustrates, we assume that the nanoscopic lipid vesicles associate spontaneously with the charges at the microgel surface. Once the surface of microgels is covered, the lipid vesicles probably spread open and form a lipid bi (or multi)layer.

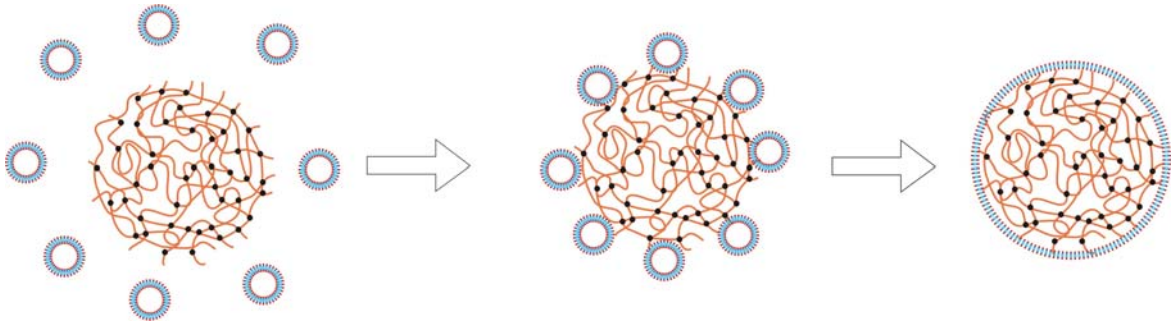

Figure 3. Adsorption of charged lipid vesicles onto oppositely charged dex-HEMA microgels. The black dots represent the cross-links in the dex-HEMA microgels.

Besides CSLM also electrophoretic mobility measurements proved that the dex-HEMA gels became coated. As Figure 5 shows, the zeta-potential of negatively and positively charged dex-HEMA microgels turns respectively positive and negative upon exposing them to the oppositely charged lipid vesicles.

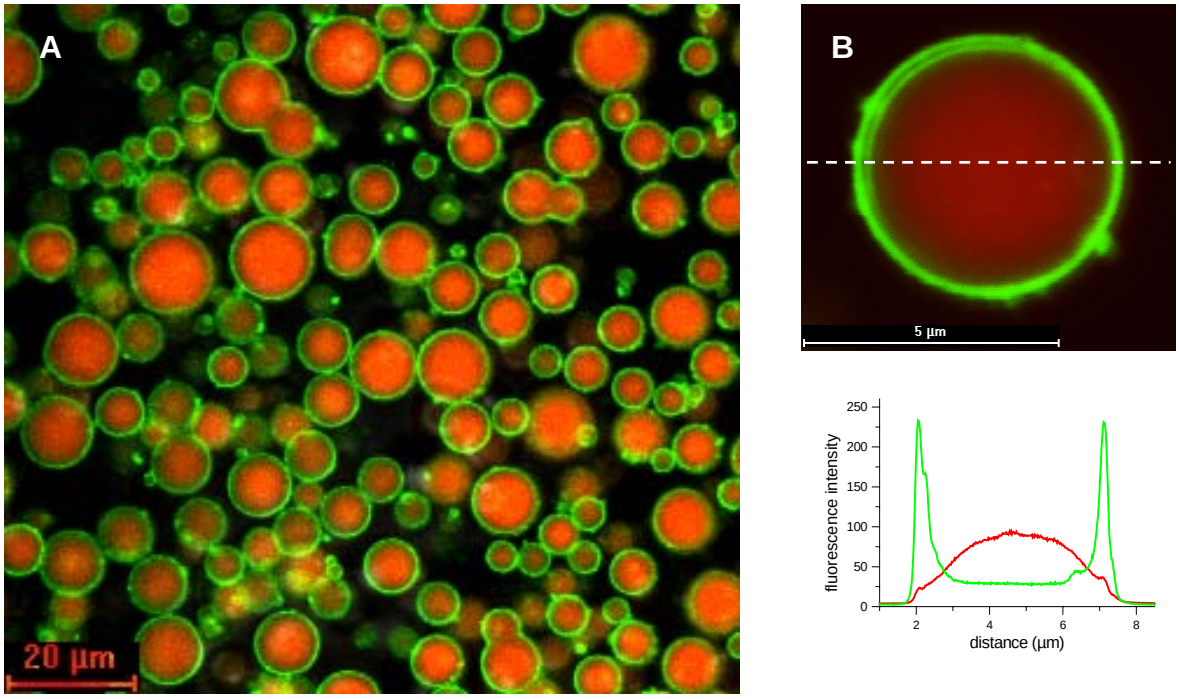

Figure 4A. CSLM images of dex-HEMA-MAA microgels coated with SOPC:DOTAP (9:1). The core is red as the microgels are fluorescently labeled with TRITC-dextran and the coat is green as the lipid layer is labeled with cholesteroyl BODIPY-FL C_{12}. The coating procedure seems very efficient as all microgels seems to be lipid coated. **B.** Red and green fluorescence intensity along the line indicated in the lipid coated microgel above.

Figure 5. ζ-potential of uncoated and lipid coated microgels. **A.** Negatively charged dex-HEMA-MAA gels coated with the positively charged lipid SOPC:DOTAP (9:1). **B.** Positively charged dex-HEMA-DMAEMA gels coated with the negatively charged lipid SOPC:DOPA (9:1).

CLSM does not give any information on the morphology of the (lipid coated) microgels, therefore scanning electron microscopy was used to itemize the fine structure of the surface of the (non) coated microgels. Figure 6 despicts SEM images of repectively non coated (left image) and coated microgels (right image). It reveals that the surface of the uncoated microgels is smoother compared to the surface of the coated microgels.

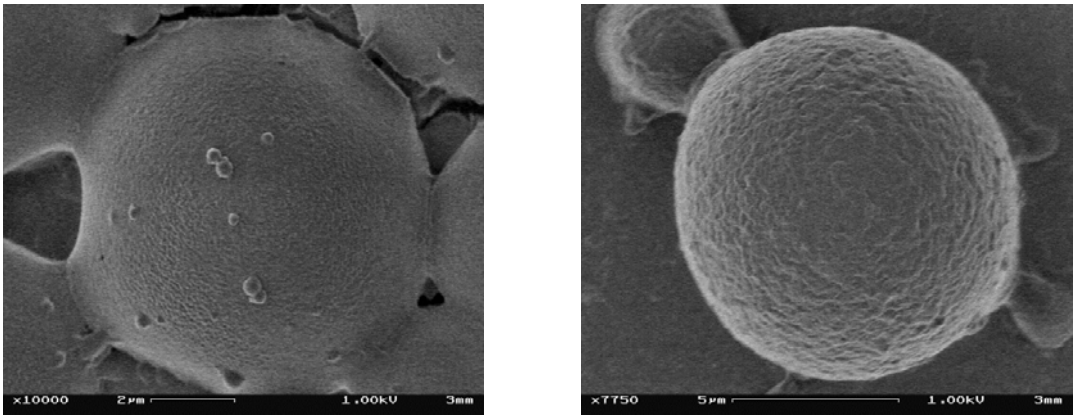

Figure 6. SEM images of uncoated dex-HEMA-MAA microgels (left) and SOPC:DOTAP coated dex-HEMA-MAA microgels (right).

Tensile strength of the coating and swelling pressure of the gel core

As described above we coated the dex-HEMA microgels with lipids. One argumentation to use lipids is that they are biocompatible and that liposomes are already medically used. Another major reason however is that we found out that degrading dex-HEMA microgels have a reasonable chance to rupture a surrounding lipid coating. These findings followed from the considerations below.

First, we determined the osmotic pressure gradient necessary to rupture the lipid vesicles used to coat the dex-HEMA microgels. Therefore, CF containing (uni-lamellar) SOPC:DOTAP (9:1) lipid vesicles (100 nm) were bathed in buffered NaCl solutions with different osmotic activities. Figure 7 shows the CF release. Little CF is released if the osmotic pressure (osmotic activity) gradient is lower than about 1250 mOsm while significant CF release occurs at higher osmotic pressure gradients. The intersection with the x-axis of the linear fit of the data points with a CF release higher than 10%, estimates the average maximum osmotic pressure gradient the lipid vesicles can withstand.[25,26,31] When vesicles are exposed to gradients lower than this value they probably only swell causing dilational strains of the membrane which results in a higher membrane tension. At the maximal membrane tension elastic behavior terminates in membrane rupture and lysis occurs. Clearly, the CF release is not an ‘all-or-nothing’ effect. The reason why the CF

release does not occur suddenly at a certain value of the osmotic gradient but gradually has been debated by others.[26] It is attributed to both the size distribution of the vesicles (the smaller vesicles tolerating much greater osmotic pressure gradients than the larger vesicles, as predicted by Laplace law) and the so called 'membrane resealing' whereby the vesicles release only a portion of their content.

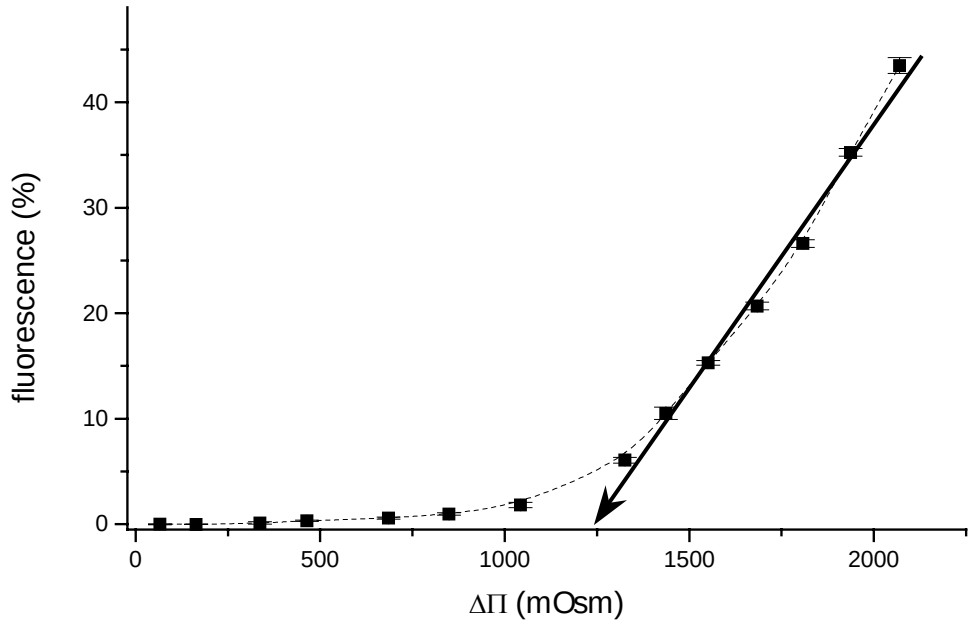

Figure 7. Release of CF from (uni-lamellar) SOPC:DOTAP (9:1) vesicles (100 nm) diluted in NaCl containing buffer solutions. The x-axis represents the osmotic pressure gradient being the difference between the osmotic activity of the CF solution in the vesicles ('internal solution') and the osmotic activity of the NaCl buffered solution ('external solution'). Compared to the internal solution the external solutions are hypo-osmotic. The y-axis represents the fluorescence of the external solution (100% being the fluorescence of the external solution after complete lysis of the vesicles by Triton X-100). The measurements were performed in triplicate.

Second, knowing the osmotic pressure gradient necessary to rupture the uni-lamellar SOPC:DOTAP (9:1) lipid membrane of 100 nm vesicles, using Laplace's law one can calculate the tensile strength of the membrane when it ruptures, being the maximal tensile strength (τ_{max}) of the membrane:

$$\tau_{max} = \frac{\Delta\pi \times r}{2} \quad (1)$$

where $\Delta\pi$ (N/m^2~ mOsm) is the osmotic pressure gradient and r (m) is the radius. For the uni-lamellar SOPC:DOTAP (9:1) membrane τ_{max} of 750 N/m was found. Table I overviews τ_{max}–values we obtained for neutrally, positively and negatively charged uni-lamellar membranes made from different lipid compositions. The tensile strengths obtained are higher than the tensile strength reported by Needham et al. using the micro-aspiration technique.[32] This might be explained by differences in time course at which the experiments are performed. Table I also shows that τ_{max} of DOPC:CHOL vesicles increases with increasing cholesterol concentration. This has also been observed and explained by others. [32]

Third, assuming dex-HEMA microgels (e.g. 3 μm in diameter) become coated with a uni-lamellar lipid membrane one can consequently calculate (by equation 2) which pressure the degrading microgel has to excert to rupture the membrane in case the coated microgels are bathed in a solution which is iso-osmotic to the interior of the particles. Table I overviews the 'critical swelling pressure' ($\pi_{swell,\ crit}$) necessary to rupture lipid coated dex-HEMA microgels of 3 μm in diameter.

Fourth, $\pi_{swell,\ crit}$ is now compared with the increase in swelling pressure of dex-HEMA gels when they degrade. Dex-HEMA hydrogels degrade through hydrolysis of the carbonate ester bounds in the HEMA based crosslinks. Hydrolysis results in high molecular weight dextran chains (M_n of 19 000 g/mol) and poly-HEMA fragments (with an estimated M_n of 1300 g/mol); when totally degraded dex-HEMA gels become a dextran solution. In previous research we focused on the change in swelling pressure of degrading (neutral) dex-HEMA gels. A remarkable profile of the swelling pressure upon degradation was obtained for the dex-HEMA hydrogels. Apparently, after an initial phase in which the swelling pressure raised slightly, a steep increase in swelling pressure was obtained at the end of the degradation.[33,34] The degradation time in physiological conditions of the dex-HEMA gels can be tailored by both the degree of substitution and the concentration and can be tuned from days to several weeks. The maximal swelling pressure which can be reached upon hydrogel degradation is only determined by the initial dex-HEMA concentration. Table II shows the maximal swelling pressure which arises through degradation of different types of dex-HEMA gels. Comparing these values with $\pi_{swell,\ crit}$ (Table I) suggests that the lipid membrane surrounding 3 μm sized dex-HEMA microgels may become ruptured by the degrading dex-HEMA gel core.

Tabel I. Charge, maximal tensile strength (τ_{max}) and critical swelling pressure ($\Pi_{swell,crit}$) of lipid membranes with different compositions. τ_{max} being the maximal tensile strength the membrane can withstand. $\Pi_{swell,crit}$ being the osmotic pressure difference necessary to lyse the lipid membrane of 3 µm sized lipid coated microgels.

Lipid composition	Molar ratio	charge	τ_{max} (10^2. N/m)	$\Pi_{swell,crit}$ (kPa)
DOPC:CHOL	10 :0	0	6,8 ± 0,1	91 ± 1
DOPC:CHOL	9:1	0	7.0 ± 0,0	93 ± 1
DOPC:CHOL	7:3	0	8,5 ± 0,1	113 ± 1
DOPC:CHOL	5:5	0	9,1 ± 0,2	121 ± 2
SOPC:DOTAP	9:1	+	7,5 ± 0,0	101 ± 0
SOPC:DOTAP	7:3	+	7,8 ± 0,1	103 ± 1
SOPC:DOTAP	5:5	+	6,4 ± 0,0	86 ± 1
SOPC:DOPA	9:1	-	6,5 ± 0,1	86 ± 1
SOPC:DOPA:CHOL	10:1:4	-	6,5 ± 0,3	87 ± 5

Tabel II. Swelling pressure of different dex-HEMA hydrogel compositions at 25°C (see Figure 6 of Chapter 3)

Dex-HEMA hydrogel type	Swelling pressure (kPa)
DS 2.9; 25%	114 ± 3
DS 2.9; 30%	161 ± 3
DS 5.0; 20%	76 ± 2
DS 7.5; 20%	76 ± 2

Self-explosion of core-shell microparticles

We further examined the behavior of the lipid coating during and after complete degradation of the microgel core. As lipid membranes are known to have limited permeability, we expected that the degradation products of the dex-HEMA microgels (i.e. dextran chains M_n of 19 000 g/mol) would not leave trough the lipid membrane. Depending on the composition, complete degradation of dex-HEMA gels ranges from days to weeks, as explained above. To accelerate the degradation we submerged the lipid coated dex-HEMA microgels in an alkaline solution and followed them in time by CLSM (Figure 8). The microgels first swoll moderately; At a given moment a huge volume expansion occurred followed by a destruction of the lipid coating. The lag-time in

swelling of the coated microgels was somehow in agreement with the lag-time in swelling pressure we observed earlier for degrading dex-HEMA gel slabs.[33,34] As a 'negative control' we also followed the behavior in time of non-coated dex-HEMA microgels. The picture was totally different: the microgels seemed to dissolve gradually in the alkaline solution (data not shown as clear images of the microparticles could not be obtained during the degradation experiment).

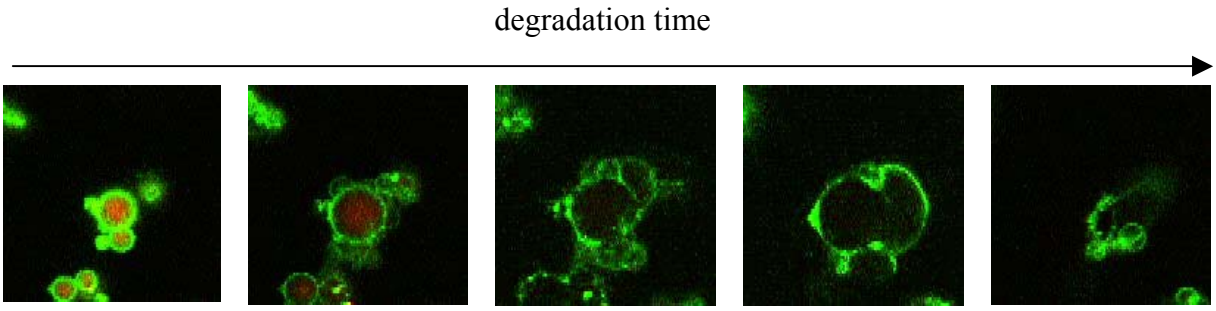

Figure 8. CLSM images of SOPC:DOTAP (9:1) coated dex-HEMA-MAA microgels bathed in an alkaline solution. Snapshots were taken every 15 sec. In some particles localized areas of expansion at the surface of the microgels were visible. Possibly due to heterogenieties in the lipid coating certain areas of the microgel seemed to swell stronger before the entire particle explodes. Similar observations were seen by Kraft et al. on lipid coated microgels exposed to detergents.[35]

To prove that the alkaline solution did not damage the lipid membrane we prepared lipid coated (SOPC:DOTAP (9:1)) dextran-methacrylate (dex-MA) hydrogels containing MAA. While dex-HEMA hydrogels degrade through hydrolysis, dex-MA gels do not as carbonate esters are not present in the cross-links. Non-coated dex-MA gels were stable in alkaline solutions and did not swell. Also, lipid coated dex-MA-MAA microgels bathed in alkaline solution did not swell while the lipid coating remained intact (data not shown). This allowed us to conclude that the rupture of the membrane in the lipid coated dex-HEMA microgels is attributed to the increase in swelling pressure of the degrading hydrogel core.

CONCLUSIONS

This study shows that charged dex-HEMA microgels can become lipid coated by electrostatic interaction with oppositely charged lipid vesicles. From CLSM experiments we concluded that microparticles with a 'core-shell' structure were obtained. Especially,

the coating method seemed to be very efficient as all dex-HEMA microgels became surrounded by a lipid membrane. SEM measurements revealed a rougher surface of the coated microgels compared with he surface of non-coated microgels. The maximal tensile strength lipid membranes can withstand (τ_{max}) was further determined. From τ_{max} we calculated the swelling pressure the gel core (assuming 3 μm in diameter) needs to rupture its surrounding lipid coating. These calculations showed evidence that rupturing the lipid membrane by the degrading microgels should be possible. Indeed, 'explosion' of the lipid coating could be confirmed experimentally.

ACKNOWLEDGEMENTS

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SUMMARY

Pharmaceutical research strives to design drug delivery systems that respond to therapeutic needs. Because of rhythms in physiological parameters and pathological conditions (e.g. asthma, angina pectoris), the conventional paradigm concerning drug concentrations “the flatter the better” may not be what the organism may need. Instead, to correlate with our biological needs, 'precisely timed drug delivery', which can be accomplished with ‘programmable dosage forms’, is required. Precisely timed drug delivery may maximize therapeutic efficacy, may minimize dose frequency and may reduce toxicity by avoiding side effects and drug tolerance. It was our intention to use hydrogels from (hydroxyethyl) methacrylated dextrans (dex-(HE)MA) in this study for creating such a programmed dosage form. Concepts proposed to release drugs in a pulsed manner from ‘programmed polymeric pharmaceutical devices’ were reviewed. The devices were classified based on the physicochemical and biological principles that trigger the release: (i) pulsed delivery by degradation of the device (like spontaneous hydrolysis and enzymatic degradation) (ii) pulsed delivery by osmotic pressure of the device (i.e. by osmotic pumping and by osmotic bursting) and (iii) pulsed delivery by the combination of degradation and osmotic bursting of the device. In the last part of the literature overview our concept of ‘exploding coated microspheres’, consisting of a degradable hydrogel core and a semi-permeable membrane is introduced *[see Figure 1, Chapter 3]*. The membrane is only permeable to water and not permeable to the entrapped drugs or osmotic agents. However, after drug administration by injection (simulated in vitro by immersion in buffer), water permeates through the membrane in the hydrogel containing the drug, the hydrogel core degrades and the internal osmotic pressure of the membrane surrounded hydrogel rises until the membrane is ruptured. At that moment, after a well defined lag-time, the drugs are liberated from the hydrogel core. The combination of different types of degradable coated microgels could be of particular use in e.g. ‘single shot vaccination’ in which the initial and subsequent booster release of antigens could be obtained in one single injection. The general aim of this study was to design ‘exploding coated microspheres’ as pulsed delivery system by using membrane surrounded hydrogels. As core material dextran based hydrogels were used. As membrane we especially focused on lipids. In order to identify the key parameters that influence the bursting of the semi-permeable membrane and drug release, it was decided to study two main aspects: (i) the thermodynamic properties of the degrading hydrogels (such as the above described hydrogels) and (ii) the mechanical properties of the membrane (such as lipids).

The thermodynamic properties include the swelling pressure (Π_{sw}) of the hydrogel which is described as the sum of two terms: an osmotic pressure (Π_{osm}) expanding the network, and an elastic pressure (Π_{el}) acting against expansion. Since membrane surrounded degrading hydrogels develop a pressure as a function of time, it was likely that these properties somehow correlated with the rupture of the membrane followed by drug

release. Therefore, an in depth analysis of the thermodynamic properties of the hydrogels was performed, to gather enough information to describe the evolution of the swelling pressure during hydrogel degradation.

Because hardly any references were found dealing with swelling pressures of (degrading) hydrogels, a methodology was developed. This method based upon osmotic deswelling, allowed measuring the swelling pressure of degrading and non-degrading dex-HEMA hydrogels. It is found that the degradation rate strongly depends on the initial dex-HEMA concentration and degree of substitution (DS , number of hydroxyethyl methacrylates per 100 glucopyranose residues). The variation of the swelling pressure at each stage of degradation could be satisfactorily described by the equation $\Pi_{sw} = A(\varphi^{n} - \varphi_{e,t}^{n-1/3}\varphi^{1/3})$, where $\varphi_{e,t}$ is the concentration of the fully swollen gel at degradation time t, and *A* and *n* are constants. A remarkable profile of the swelling pressure upon degradation was obtained for the dex-HEMA hydrogels *[see Figure 2B, Chapter 3]*: apparently, after an initial phase in which the swelling pressure raised slightly, a steep increase of the swelling pressure was obtained at the end of the degradation. The slight changes in swelling pressure during the first 15 days of degradation is caused by the decrease of the elastic pressure. The end of the degradation is accompanied by the release of a major amount of dextran chains. The sudden increase in swelling pressure at the end of the degradation can be explained by the fact that only at the end of the degradation (close to the gel-sol transition) the free dextran chains contribute to the osmotic pressure. Before the dextran chains behave as additional polymer network chains and remain physically entrapped.

We wondered whether we could confirm the remarkable swelling pressure profiles of the degrading dex-HEMA gels by using dex-HEMA gels containing different amounts of free dextran. It was concluded that dex-HEMA gels made in the presence of known amount of free dextran chains exhibit osmotic properties similar to those of the partially degraded dex-HEMA gels *[see Figure 4, Chapter 3]*. To develop degradation controlled exploding microspheres as platform for pulsed drug delivery, it was desirable to design hydrogels that possess different degradation kinetics. Swelling pressure versus degradation time plots were obtained from the swelling pressure measurements of dex-HEMA/dextran gels in conjunction with dextran release data of degrading dex-HEMA gels. The swelling pressure profiles indicate that the profile strongly depends on the concentration of the cross-linked dex-HEMA and its degree of substitution *[see Figure 6, Chapter 3]*. The initial value of the elastic modulus, the height of the 'plateau' region of the osmotic pressure plot and the maximal reachable swelling pressure increase with increasing dex-HEMA concentration. The DS increases the amount of possible cross-links which is reflected in the increased elastic modulus. Generally increasing the cross-link density (due to increasing ether DS or dex-HEMA concentration) lengthens the degradation time and, consequently, the time required to attain a certain swelling pressure.

Because the osmotic deswelling method developed in Chapter 2 is very time and product consuming, an osmometer was developed for the charaterization of the

thermodynamic properties of degrading hydrogels *[see Figure 2, Chapter 4]*. Degradation induced changes in the osmotic swelling pressure of pharmaceutical hydrogels are measured by confining the hydrogel in an enclosure between a rigid semi-permeable membrane and the diaphragm of a pressure transducer. Sensor response time ranged between 3 and 6 hours for polymer solutions and polymer hydrogels respectively. The device was validated by measurement the pressure of dextran and PEG solutions with known osmotic pressure. The pressure values were in good agreement with literature data. In order to further validate the device the swelling pressure of a dex-MA (DS3.1; 25%) hydrogels was measured and compared with osmotic deswelling data of the same gel. It takes about 64 ± 10 kPa of mechanical pressure and 69 kPa of osmotic stress to keep the hydrogel volume fixed. Therefore both methods are considered to be in acceptable agreement. The device is able to monitor constant changes in osmotic properties of the enzymatic degrading hydrogel, i.e. dex-MA/dextranase gel. However, degradation of the dex-HEMA gels was inhibited inside the device and changes in osmotic properties could not be followed. In conclusion, depending on the type of hydrogel system and depending on its degradation mechanism the home-made osmometer is a suitable alternative for the osmotic deswelling technique.

Having established good methods for evaluation of the swelling pressure of degrading hydrogels, the influence of the degradation mechanism on the swelling pressure was analysed in more detail. A hydrogel can be bulk degraded by two different mechanisms: by a degradation of the polymer backbone or by a degradation of the cross-links. As a model for hydrogels which are degraded at the polymer backbone, dex-MA hydrogels with incorporated dextranase (dex-MA/dextranase hydrogels), which hydrolyses the dex-MA chains, were used. The degradation of hydrogels at the cross-links was studied using dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels. The elastic moduli, the release of the degradation products, the swelling and swelling pressure of the degrading dextran-based hydrogels were measured. The release of degradation products, being oligosaccharides in dex-MA/dextranase gels and dextran chains in dex-HEMA gels, and especially the swelling pressure profile seems to be strongly dependent on the degradation mechanism of the gels. In case the dextran gels are degraded at their backbone the swelling pressure increases rather continuously *[see Figure 4, Chapter 5]*, in case they are degraded at the cross-links it increases more discontinuously as a sudden increase occurs when the gels are (nearly) completely degraded. This study reveals that the increase in swelling pressure in degrading dex-MA/dextranse gels is (nearly) completely attributed to an increase in osmotic pressure. However, in degrading dex-HEMA gels the increasing swelling pressure seems to be attributed to the decrease in elastic pressure (i.e. elasticity) of the gels. Indeed, during a substantial period the osmotic pressure of degrading dex-HEMA gels does not change. At complete degradation the swelling pressure equals the osmotic pressure of the solution of degradation products which is much higher when the gels are degraded at their backbone than when they are degraded at their cross-links.

Using this steep increase in swelling pressure of degrading dex-HEMA gels to rupture a membrane is ideal because it restricts the broadening of the release pattern due to size distributions of the microgels and inhomogenities in the surrounding membrane. These effects would dramatically influence the release pattern when for example a gradual increase of swelling pressure would occur as is the case for the dex-MA/dextranase hydrogels.

The mechanical properties of the membrane surrounding the hydrogel core was chosen as second key aspect in this work, since it also determines possibility of rupture of the membrane. Therefore, it was wondered which membrane should be used in order to obtain a unique membrane surrounded hydrogel delivery system which is able to release drugs due to osmotic bursting of the membrane due to hydrogel degradation. Lipids were chosen to construct the membrane due to their similarity with natural membranes, which have the ideal properties for our proposed pulsed delivery system (i.e. highly water permeable and restricted permeability for drugs and hydrogel degradation products). We measured the tensile strength by following the carboxyfluorescein released from different lipid vesicles due to osmotic induced lysis. Using the law of Laplace, the internal pressure needed to rupture 3 µm lipo-beads ranged between 85 and 120 kPa. As a swelling pressure from 75-150 kPa can be reached by degrading dex-HEMA gels, it is concluded that the hydrogels should theoretically be able to rupture the lipid membrane. Lipid coating of hydrogels is not straightforward. We used respectively positively and negatively charged gels by adding DMAEMA or MAA during gelation and oppositely charged lipids (i.e. SOPC:DOPA and SOPC:DOTAP). By using electrostatic interaction between the oppositely charged gel and membrane we obtained a nice core-shell structure and a yield of 100% lipid coated microgels *[see Figure 4, Chapter 6]*. The coating of the microgels was evidenced by measuring the electrophoretic mobility, confocal laser scanning microscopy and scanning electron microscopy. Finally, as cherry on the pie, the proof of principle is given. Exploding coated microspheres i.e. lipo-beads exist *[see Figure 8, Chapter 6]*! To visualize the degradation we observed with CLSM the behavior of the coated and non-coated microgels when they were added to a solution of NaOH. In both cases the microgels started to swell moderately and at a given moment this swelling accelerated. In case of non-coated microgels, they dissolve completely and no traces remain. In case of coated microgels, the swelling of continues and at a sudden moment an explosion of the surrounding lipid membrane occurs, leading to the release of the encapsulated material. By using lipid coated non-degradable dex-MA-DMAEMA microgels, it was proven that the NaOH did not damaged the lipid membrane and that the bursting of the lipo-beads indeed was caused by the increased internal pressure due to microgel degradation.

SAMENVATTING

Farmaceutisch onderzoek streeft ernaar om formulaties te ontwikkelen met een geneesmiddelen vrijstelling die voldoet aan de therapeutische noden. Door fluctuaties in fysiologische parameters en pathologische condities (zoals astma en angina pectoris) kan het zijn dat de gebruikelijke doseringsschema's waarbij een constante geneesmiddelen concentratie beoogt wordt gedurende een bepaalde tijd niet echt voldoet aan de noden van ons lichaam. Om te voldoen aan onze biologische noden kan tijdsgecontroleerde geneesmiddelen vrijstelling met behulp van specifieke formulaties noodzakelijk zijn. Voordelen van gepulseerde geneesmiddelen vrijgave zijn: (i) verhoogde therapeutische efficiëntie, (ii) verlaagde doseringsfrequentie en (iii) verlaagde toxiciteit door neveneffecten en geneesmiddelen tolerantie te vermijden.

In deze thesis was het onze bedoeling om hydrogelen van (hydroxyethyl) gemethacryleerd dextraan (dex-(HE)MA) te bestuderen met het oog op de ontwikkeling van een dergelijke formulatie voor tijdsgecontroleerde geneesmiddelen vrijgave. Een literatuur overzicht in verband met geneesmiddelen vrijstelling uit 'farmaceutische geprogrammeerde formulaties op basis van polymeren' werd gegeven. De verschillende formulaties werden als volgt geklasseerd: (i) gepulseerde vrijstelling door de afbraak van de matrix (zowel hydrolytisch als enzymatisch); (ii) gepulseerde vrijstelling door het ontstaan van een inwendige osmotische druk en (iii) gepulseerde vrijstelling door de combinatie van enerzijds afbraak van de matrix en anderzijds osmotische vernietiging van de formulatie. Op het einde van het literatuur overzicht werd ons concept van 'exploderende micropartikels' geïntroduceerd *[zie Figure 1, Chapter 3]*. Exploderende micropartikels bestaan uit een degraderende hydrogel kern die omgeven wordt door een semi-permeabele membaan. Deze membraan is enkel doorgankelijk voor water en niet voor de ingesloten geneesmiddelen en de osmotische agentia (namelijk de afbraakproducten). Na injectie van de gecoate microsferen (gesimuleerd in vitro door immersie in buffer), diffundeert het water doorheen de semi-permeabele membraan. Daardoor degradeert de geneesmiddelen bevattende hydrogel kern en ontstaat er een inwendige osmotische druk in de kern. Wanneer de inwendige osmotische druk groter wordt dan de treksterkte van de membraan, zal de membraan barsten (exploderen). Op dat ogenblik zullen de geneesmiddelen vrijgesteld worden. Injectie van meerdere exploderende microsferen met verschillende degradatie karakteristieken lijken ideaal voor vele toepassingen zoals bijvoorbeeld éénmalige vaccinatie, waarbij zowel de initiële als de booster vrijstelling gelijktijdig geïnjecteerd wordt. Het was dan ook de algemene doelstelling van dit werk om door middel van een gecoate hydrogel een exploderend partikel te ontwikkelen, die in staat is zijn geneesmiddelen op een gepulseerde manier vrij te stellen. Dextraan hydrogelen werden bestudeerd als kern materiaal. Als membraan ging onze aandacht uit naar lipiden omwille van hun verwantschap met de celmembraan. Om inzicht te krijgen in de parameters die het barsten van de membraan en bijhorende geneesmiddelen vrijstelling veroorzaken, werd gekozen om het onderzoek toe te spitsen

op twee onderwerpen: (i) de thermodynamische eigenschappen van de degraderende hydrogelen (zoals de hierboven vermelde dextraan gelen) en (ii) de mechanische eigenschappen van de membraan (zoals de lipiden).

Als thermodynamische parameter werd de zweldruk geëvalueerd van de hydrogel kern. De zweldruk bestaat uit een som van twee termen: de osmotische druk die expansie van het netwerk veroorzaakt en de elastische druk die deze expansie tegenwerkt. Aangezien een membraan omgeven degraderend hydrogel een druk zal opbouwen in functie van de tijd, is het vrij duidelijk dat deze eigenschappen bepalend zijn voor het barsten van de membraan. Om voldoende inzicht te krijgen in de evolutie van de zweldruk die optreedt tijdens hydrogel degradatie, werd een diepgaande analyse van de thermodynamische eigenschappen van de degraderende hydrogelen uitgevoerd.

Tot onze verbazing waren er geen referenties te vinden die de karakterisatie van de zweldruk van degraderende hydrogelen gedetailleerd beschreven. Er werd dan ook besloten zelf een methode te ontwikkelen. De methode is gebaseerd op osmotische ontzwelling en laat toe de zweldruk van een al dan niet degraderend hydrogel te bepalen. Dex-HEMA hydrogelen met verschillende samenstelling werden aangemaakt door de dex-HEMA concentratie en de substitutiegraad van het dex-HEMA (DS, aantal hydroxyethyl methacrylaatgroepen per 100 glucopyranose eenheden) te variëren. Uit reologische metingen en bepalingen van de suiker vrijstellingsproducten bleek dat de degradatie kinetiek van de hydrogelen sterk afhankelijk is van de gebruikte samenstelling. Het effect van de degradatie op de zweldruk werd bestudeerd op de dex-HEMA hydrogel met de kortste degradatietijd (namelijk dex-HEMA DS2.9; 25%). Op ieder ogenblik van de degradatie kon de evolutie van de zweldruk beschreven worden door de volgende vergelijking $\Pi_{sw} = A(\varphi^n - \varphi_{e,t}^{n-1/3}\varphi^{1/3})$, waarbij $\varphi_{e,t}$ de concentratie is van de gezwollen gel op tijdstip t, en *A en n* constanten zijn. Een heel opmerkelijk zweldruk profiel werd bekomen *[zie Figure 2B, Chapter 3]*. Na een initiële fase waarin de zweldruk slechts weinig toenam, volgde op het eind van de degradatie een plotse en sterke stijging in zweldruk. De lag fase in de zweldruk gedurende de eerste 15 dagen van de dex-HEMA degradatie kan verklaard worden door de daling in elastische druk. De plotse toename in zweldruk op het einde van de degradatie kan verklaard worden door het feit dat alleen op het einde van de degradatie (dicht bij de ‘gel-sol transitie’) de vrije dextraan ketens bijdragen tot de osmotische druk. Vóór de ‘gel-sol transitie’ gedroegen de dextraan ketens zich als additionele polymeer netwerk ketens en bleven ze als het ware fysisch gevangen in de gel (het netwerk).

We vroegen ons af of we dit opmerkelijk zweldruk profiel konden bevestigen door de degraderende dex-HEMA gels na te bootsen met behulp van niet gedegradeerd dex-HEMA gels die verschillende hoeveelheden vrij dextraan (afbraakproduct) bevatten. En inderdaad, dex-HEMA hydrogelen die een gekende hoeveelheid vrij dextraan bevatten vertoonden gelijke osmotische eigenschappen als gedeeltelijk gedegradeerde dex-HEMA gels *[zie Figure 4, Chapter 3]*. Om de degradatie gecontroleerde exploderende microsferen te gebruiken voor meervoudige gepulseerde geneesmiddelen vrijgave is het interessant de zweldrukken van hydrogelen met verschillende

degradatie kinetiek te kennen. Zweldruk versus degradatie tijd grafieken werden bekomen door de zweldruk profielen van de dex-HEMA/dextraan hydrogelen te combineren met dextraan release curven van de degraderende dex-HEMA gelen. Zoals verwacht, waren de bekomen zweldruk profielen sterk afhankelijk van zowel de gebruikte dex-HEMA concentratie als van de gebruikte substitutiegraad *[zie Figure 6, Chapter 3]*. De initiële waarde van de elastische druk, de hoogte van het plateau van de osmotische druk en de maximale zweldruk (op het einde van de degradatie) stegen met toenemende dex-HEMA concentratie. Toename van de substitutiegraad resulteerde in een hoger aantal knooppunten wat gereflecteerd werd in een hogere initiële elasticiteits modulus. Een toename in knooppunten (door toename van DS en/of concentratie) resulteerde bovendien ook in een toename van de degradatie tijd en bijgevolg ook in de toename van tijdsduur vooraleer een welbepaalde zweldruk werd bereikt.

Omdat de osmotische ontzwellingsmethode ter bepaling van de zweldruk van degraderende hydrogelen enorm tijdsrovend is en veel materiaal verbruikt, werd er een osmometer ontwikkeld voor de bepaling van de thermodynamische eigenschappen van de bestudeerde dex-HEMA gels *[zie Figure 2, Chapter 4]*. De veranderingen in zweldrukken van de farmaceutische hydrogelen die optreden tengevolge van degradatie worden gemeten door een hydrogel te fixeren tussen een rigide semi-permeabele membraan en het diafragma van een druk transducer. De reactie tijd van de sensor bedroeg 3 uren voor polymeer oplossingen en 6 uren voor gels. Het toestel werd gevalideerd met behulp van polymeer oplossingen (dextraan en poly(ethylene)glycol (PEG)) met gekende osmotische druk. Zowel voor de PEG-oplossingen als voor de dextraan oplossingen kwamen de gemeten data overeen met literatuur gegevens. In een volgende stap werd de zweldruk gemeten van een dex-MA (DS3.1; 25%) gel in de osmometer en anderzijds ook door middel van osmotische ontzwelling. Ongeveer 64 ± 10 kPa mechanische druk en 69 kPa osmotische druk was noodzakelijk om het volume van de hydrogel constant te houden. Bijgevolg konden we concluderen dat beide methoden om zweldrukken te meten vergelijkbaar resultaat opleveren. In een laatste stap werd het toestel geëvalueerd voor de meting van de zweldruk van degraderende hydrogelen. Hiervoor werd zowel een enzymatisch degraderend hydrogel nl. dex-MA/dextranase alsook een chemisch hydrolyserend hydrogel uitgekozen. De veranderingen in osmotische eigenschappen van de dex-MA/dextranase gelen konden gemakkelijk gevolgd worden. Maar spijtig genoeg was dat niet het geval voor de dex-HEMA hydrogelen. We kunnen dus stellen dat voor het meten van de zweldruk van degraderende hydrogelen, zowel de samenstelling (cfr. gels die krimpen kunnen niet gemeten worden in de osmometer) als het mechanisme van degradatie van de gel bepalend is of de osmometer een geschikt alternatief vormt voor de osmotische ontzwellingsmethode.

Nadat een goed inzicht verkregen werd in de methoden om zweldrukken te evalueren van degraderende hydrogels werd een studie opgezet omtrent hun degradatie. De invloed van het degradatiemechanisme werd geëvalueerd. Er zijn twee degradatiemechanismen mogelijk voor de bulk degradatie van een hydrogel: degradatie

van het polymeer (de ruggengraat van het netwerk) en degradatie van de knooppunten. Dex-MA hydrogelen kunnen worden afgebroken door dextranase in te sluiten tijdens de gelering van de dex-MA oplossingen. Dextranase knipt in de dex-MA ketens. Om afbraak door degradatie van de knooppunten te evalueren werden dextraan hydroxyethyl methacrylaat (dex-HEMA) hydrogelen onderzocht. Deze hebben een hydrolyseerbare groep ter hoogte van de knooppunten. Zowel de elasticiteit, de vrijstelling van de degradatie producten, de zwelling en de zweldruk van de hierboven vermelde hydrogelen werden gemeten. De studie toonde dat het degradatiemechanisme vooral een grote invloed heeft op de vrijstelling van de degradatie producten (oligosacchariden voor dex-MA /dextranase gels en dextraan in het geval van dex-HEMA gels) en op het zweldruk verloop. In het geval van de dex-MA/dextranase gels (degradatie ter hoogte van de ruggegraat) steeg de zweldruk eerder continu *[zie Figure 4, Chapter 5]*. In het geval van de dex-HEMA gels (degradatie t.h.v de knooppunten) hebben we een discontinu verloop van de zweldruk dat gekenmerkt werd door een plotse stijging in zweldruk op het einde van de degradatie. We toonden aan dat de stijging in zweldruk van de dex-MA/dextranase gels bijna volledig te wijten is aan de toename in osmotische druk gedurende de dex-MA degradatie. Voor de degraderende dex-HEMA gels kon het zweldruk profiel initieel verklaard worden door een daling in elastische druk, aangezien gedurende een substantiële periode van de degradatie de osmotische druk niet veranderde. Bij volledige degradatie is de zweldruk gelijk aan de osmotische druk van de oplossing met degradatie producten. Gezien het grote verschil in molecuul gewicht van de afbraakproducten van beide gelen, was het vanzelfsprekend dat de finale druk veel hoger was voor de dex-MA gels dan voor de dex-HEMA gels.

Het opmerkelijk zweldruk profiel van de degraderende dex-HEMA gels is ideaal om een membraan te laten barsten. De plotse toename in zweldruk op het einde van de degradatie zal de tijdsduur van geneesmiddelen vrijgave beperken die zou kunnen veroorzaakt worden door niet homogene eigenschappen van de membraan en variatie in de microgel grootte. In de rest van dit werk werd dus geopteerd om dex-HEMA microgelen als kernmateriaal van onze exploderende microsferen te gebruiken.

Aangezien het slagen van ons concept niet alleen bepaald wordt door de thermodynamische eigenschappen van de kern maar ook door de mechanische eigenschappen van de membraan, werd in een volgende stap de membraan bestudeerd. We stelden ons de vraag welke membraan zou resulteren in een uniek membraan omgeven hydrogel systeem, dat zijn geneesmiddelen plots vrijgeeft door het barsten van de membraan ten gevolge van de osmotische druk, opgebouwd tijdens hydrogel degradatie. Lipiden bezitten ideale eigenschappen zoals een voldoende water permeabiliteit en een beperkte permeabiliteit voor geneesmiddelen en osmotische agentia. De treksterkte van de membranen werd geëvalueerd door de carboxyfluoresceïne vrijgave uit lipid vesikels met verschillende samenstellingen te bepalen. De carboxyfluoresceïne vrijgave trad op ten gevolge van een osmotische gradient tussen de vesikel inhoud en de omgevingsvloeistof die resulteerde in vesikel lysis. Gebruik makend van de wet van Laplace, konden we

berekenen dat voor 3 µm lipo-gels (lipid gecoate microgelen) de inwendige druk nodig voor een breuk in de membraan, tussen de 85 en 120 kPa lag, afhankelijk van de lipide samenstelling. Aangezien door degradatie van dex-HEMA hydrogelen een zweldruk van 75-150 kPa kon bereikt worden, konden we besluiten dat, theoretisch gezien, de degraderende dex-HEMA gels in staat zouden zijn de bestudeerde lipid membranen te doen scheuren. Het leggen van een lipid membraan op microgelen was echter niet evident. Enkel door introductie van DMAEMA en MAA, waardoor respectievelijk positief en negatief geladen dex-HEMA microgelen verkregen werden, konden we lipid gecoate microgelen bekomen. De lipid coating is gebaseerd op elektrostatische interactie tussen de geladen microgels en de tegenovergesteld geladen lipid membraan (SOPC:DOPA en SOPC:DOTAP). De coating van de microgels werd gevisualiseerd met behulp van confocale laser scanning microscopie en bevestigd door middel van zeta-potentiaal metingen en elektronen microscopie. 100% coatingsefficiëntie en een mooie core-shell structuur werden bekomen *[zie Figure 4, Chapter 6]*. In een laatste stap, als kers op de taart, werd de ‘proof of principle’ geëvalueerd. Exploderende microsferen, namelijk de lipide gecoate microgelen bestaan *[zie Figure 8, Chapter 6]*! Met behulp van CLSM werd de degradatie van zowel gecoate als niet-gecoate microgelen gevolgd. Om de hydrolyse van het dex-HEMA de versnellen werden ze ondergedompeld in een 0.5 M NaOH oplossing. Zowel de gecoate als niet gecoate microgelen begonnen te zwellen, de zwelling nam toe naar het einde van de degradatie. De niet gecoate microgels vloeiden open in de omgevingsvloeistof. De gecoate microgelen daarentegen vertoonden plotseling een explosie (een barsten) van de lipide membraan met vrijstelling van de inhoud! De explosie zou kunnen veroorzaakt worden door de aanwezigheid van het NaOH. Daarom werden gecoate en niet gecoate dex-MA hydrogelen gevolgd onder de CLSM in identieke omstandigheden. De dex-MA hydrogelen degradeerden niet in aanwezigheid van NaOH. Bovendien werd er na geruime tijd geen visuele schade van de lipide membraan vastgesteld. We kunnen dus besluiten dat exploderende lipide gecoate microgelen bestaan en dat die explosie veroorzaakt werd door de inwendige osmotische druk die ontstond ten gevolge van de microgel degradatie.

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